

# Genetic evidence that apolipoprotein E4 is not a relevant susceptibility factor for cholelithiasis in two high-risk populations

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**Abstract** Apolipoprotein E (apoE) isoforms are genetic determinants of interindividual variations in lipid metabolism. To assess whether apoE is a genetic risk factor for cholesterol gallstone disease (GD), we analyzed apoE variants in populations from Chile and Germany, two countries with very high prevalence rates of this disease. ApoE genotypes were determined in Chilean gallstone patients (n = 117) and control subjects (n = 122) as well as in German gallstone patients (n = 184) and matched controls (n = 184). In addition, we studied apoE variants in subgroups of Chilean patients with strong differences in their susceptibility to acquire gallstones: 50 elderly subjects without gallstones in spite of well-known risk factors for this disease (gallstone-resistant) and 32 young individuals with gallstones but without risk factors (gallstone-susceptible). Furthermore, correlation analysis of apoE genotypes with cholesterol crystal formation times, biliary cholesterol saturation index (CSI), and gallstone cholesterol contents was performed in 81 cholecystectomized patients. In this study analyzing the largest sample set available, apoE4 genotype was not associated with an increased frequency of GD in either population. Moreover, in the Chilean population after adjusting for risk factors such as gender, age, body mass index, serum lipids, and glucose, the odds ratio for the association of the apoE4 allele and GD was significantly ( $P < 0.05$ )  $< 1$ . Also, genotypes were not correlated with cholesterol crystal formation time, CSI, or gallstone cholesterol content. **In contrast to previous smaller studies, apoE polymorphisms were not associated with susceptibility to cholesterol GD in high-risk populations.**—Mella, J. G., R. Schirin-Sokhan, A. Rigotti, F. Pimentel, L. Villarroel, H. E. Wasmuth, T. Sauerbruch, F. Nervi, F. Lammert, and J. F. Miquel. **Genetic evidence that apolipoprotein E4 is not a relevant susceptibility factor for cholelithiasis in two high-risk populations.** *J. Lipid Res.* 2007. 48: 1378–1385.

**Supplementary key words** gallstone disease • cholesterol • genetic association study

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Although the precise cause of cholelithiasis is unknown, several lines of evidence have indicated that it is a multifactorial disease in which genetic and environmental factors are involved (1–4). Differences in the prevalence of gallstone disease (GD) among ethnic groups (5–8) and recent twin and family studies (9–11) indicate that genetic factors play a major role in gallstone formation. However, common lithogenic genes contributing to GD have yet to be identified.

Cholesterol gallstones are the most frequent type of gallstones in patients from Western populations (12). Their formation is usually associated with increased cholesterol secretion into the bile and changes in biliary lipid composition (4, 13). Although the association between serum lipids and gallstones has remained controversial, decreased HDL cholesterol and increased triglyceride levels might be correlated with biliary cholesterol supersaturation and the risk for cholelithiasis (14–16). Previous studies have indicated that the interaction of sustained cholesterol supersaturation in bile with biliary proteins, such as mucins, modulates cholesterol crystallization and gallstone formation (1–4, 17–19). Together, these findings suggest that abnormalities in cholesterol and lipoprotein metabolism play an important role in the pathogenesis of cholesterol cholelithiasis.

Apolipoprotein E (apoE) plays a central role in the overall regulation of cholesterol metabolism (20, 21). ApoE, the major apolipoprotein constituent of triglyceride-rich VLDL, LDL, and chylomicron remnant particles, serves as the high-affinity ligand for the hepatic LDL receptor and

Abbreviations: apoE, apolipoprotein E; BMI, body mass index; CSI, cholesterol saturation index; GD, gallstone disease.

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the LDL receptor-related protein, which plays a critical role in the hepatic catabolism of lipoproteins (20). ApoE is encoded by a polymorphic gene located on chromosome 19, with three common codominant *APOE* alleles ( $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$ ) resulting in the substitution of arginine by cysteine at positions 112 and/or 158. Isoelectric focusing discriminates six different isoforms (E2/E2, E3/E3, E4/E4, E2/E3, E2/E4, and E3/E4) (20, 22, 23). These isoforms show differences in receptor binding affinities and catabolic rates, as reflected by serum levels and clearance rates of circulating lipoproteins (24, 25). Moreover, it has been demonstrated that the apoE polymorphism is associated with an increased risk for coronary heart disease and Alzheimer disease (26, 27).

Two previous studies suggested an association of apoE with cholesterol GD: the apoE4 isoform was associated with increased gallstone cholesterol content in cholecystectomized patients in Finland (28) and with a higher risk for gallstones in a case-control study in Spain (29). The apoE2 isoform, by contrast, was found less frequently in Finnish women with gallstones than in controls (30). However, other studies have not yielded consistent association findings (31–35). The association between gallstones and *APOE* could be attributable to higher intestinal cholesterol absorption (36, 37) and increased hepatic cholesterol uptake in apoE4 carriers (21, 25). Increased hepatic cholesterol uptake via chylomicrons represses LDL receptors (38) and HMG-CoA reductase (38). This would explain the reduction of fecal bile salt excretion observed in apoE4 carriers (35) and lead to a decreased bile salt-cholesterol ratio in bile, although no significant difference in cholesterol saturation of gallbladder bile from patients with different apoE isoforms has been reported (28, 32, 38, 39). Because *holo*-apoE is present in bile, it might alternatively, similar to apoA-I and apoA-II, have a role in the destabilization of bile and the modulation of cholesterol crystallization and stone growth.

To clarify whether apoE variants modulate susceptibility to GD, we performed a large combined genetic association (case-control) study in Chile and Germany, two countries with high-risk populations for cholesterol GD (8, 40, 41). In addition, we determined apoE polymorphisms among two selected groups of patients with extreme differences in their susceptibility to acquire gallstones: old subjects without gallstones in spite of well-known risk factors for GD and young gallstone carriers without known risk factors (8, 42, 43). Finally, to assess whether apoE polymorphisms influence bile lithogenicity, we correlated apoE genotypes and cholesterol crystal formation time, biliary cholesterol saturation index (CSI), and gallstone cholesterol content in Chilean patients subjected to cholecystectomy.

## PATIENTS AND METHODS

### Study groups

Chilean patients included in this study represent a nested case-control study based on an ongoing population-based study of the prevalence and risk factors of cholelithiasis in Chile (8). The

survey was performed in La Florida, an urban area of Santiago, which is representative of the predominant socioeconomic strata and European-Amerindian admixture of the Chilean population. We randomly sampled 2,558 adult individuals aged 18 years and older, and 1,678 subjects (66%) answered a questionnaire and attended a general health examination including abdominal ultrasonography. For the genetic study, 250 unrelated individuals [median age of 44 years (range, 18–82 years); 66 males, 168 females] with ( $n = 125$ ) and without ( $n = 125$ ) GD were randomly selected from the adult population described above. Eleven subjects were excluded because DNA was not available or PCR for apoE genotyping did not generate a product. No subjects received lipid-lowering drugs, and familial hypercholesterolemia was not detected in this cohort.

The German patients were recruited from 747 inpatients aged 18 years and older at the Department of Medicine III and the Department of Surgery, University Hospital Aachen. All patients admitted to the gastroenterology ward of the Department of Medicine III were included consecutively to exclude potential bias, and gallstone status and clinical chemical parameters were obtained on the day after admission to minimize the effects of the underlying diseases on liver metabolism and gallbladder stasis. No subjects received lipid-lowering drugs, and patients with known familial hypercholesterolemia were excluded. In the Department of Surgery, we included patients undergoing elective laparoscopic cholecystectomy only. Overall, 368 Caucasian patients (184 cases, 184 matched controls) with a median age of 64 years (range, 30–89 years) were enrolled (162 males, 206 females).

GD cases were defined as subjects who had a previous cholecystectomy for gallstones or presented cholelithiasis at the time of the study, as confirmed by ultrasound. Abdominal ultrasonography was performed by three trained operators, unaware of the subject's medical history, using real-time machines with 3.5 MHz linear transducers. The presence of gallstones was assessed according to well-accepted ultrasonographic criteria (7). Blood samples were obtained from fasted individuals for chemical analysis and DNA extraction.

To increase the chance of finding potential genetic factors involved in gallstone formation, we compared apoE genotype frequencies in two additional subgroups of Chilean patients with strong differences in their genetic susceptibility to this disease. For that purpose, we used the following selection criteria: *i*) gallstone-susceptible individuals were <30 years old, nonobese [body mass index (BMI) < 27 kg/m<sup>2</sup>], nonmultiparous female or male patients with gallstones ( $n = 32$ ); *ii*) gallstone-resistant individuals were >50 years old multiparous female or >60 years old male patients, obese (BMI > 27 kg/m<sup>2</sup>), without gallstones ( $n = 50$ ).

In addition, apoE polymorphism, gallstone cholesterol content, CSI, and cholesterol crystal formation time of gallbladder bile were investigated in a group of consecutive patients with cholesterol gallstones subjected to cholecystectomy at the Pontificia Universidad Católica Clinic in Santiago ( $n = 81$ ).

The studies were approved by the respective institutional ethical committees. Informed consent was obtained from all subjects.

### ApoE genotyping

ApoE genotyping was performed by PCR-restriction fragment length polymorphism analysis as described by Hixson and Vernier (44). We decided to use an apoE genotyping method instead of any of the apoE phenotyping methods to avoid common misclassifications, particularly of E2/E3 isoforms (45). In brief, DNA was extracted from whole blood samples (46), and apoE sequences that encompass amino acid positions 112 and 158 were amplified

by PCR using primers 5'-ACAGAATTCGCCCCGGCCTGGTACAC-3' and 5'-TAAGCTTGGCACGGCTGTCCAAGGA-3' (47). The 244 bp amplification product was digested with *HhaI* and subjected to electrophoresis on 10% polyacrylamide gels. ApoE genotypes were distinguished by the recognition of unique combinations of *HhaI* fragment sizes (44).

### Bile sampling and determination of lipids, cholesterol crystal formation time, and gallstone cholesterol content

Gallbladder bile was obtained from 81 consecutive symptomatic gallstone patients at the beginning of the cholecystectomy by needle aspiration of the gallbladder. Care was taken to avoid blood contamination of bile and to obtain a complete aspiration of the gallbladder bile to avoid the effect of stratification (48). Bile was collected in sterile tubes containing 0.05% chloramphenicol, 3 mM sodium azide, 0.2 mM thiomersal, 1 mM phenylmethylsulfonyl fluoride, and 1 mM leupeptin as preservatives. Two milliliters of bile were immediately processed for the crystal formation time assay, and the remaining bile was stored at  $-20^{\circ}\text{C}$ . Gallbladder stones were washed with distilled water, dried at  $37^{\circ}\text{C}$  for 5 days, and crushed thoroughly. Their cholesterol content was subsequently determined chemically (49). Only sterile bile specimens were included in the analysis of the data. Biliary cholesterol, bile salts, and phospholipids were quantified by standard techniques (50). CSI was calculated according to Carey's critical tables (51). Serum triglyceride, total cholesterol, LDL cholesterol, and HDL cholesterol levels were determined by standard enzymatic assays (Flex<sup>®</sup> reagent cartridges; Dade Behring, Inc., Newark, DE) on the Dimension<sup>®</sup> clinical chemistry system.

For the cholesterol crystal formation time measurement, aliquots (500  $\mu\text{l}$ ) of isotropic gallbladder bile were filtered through a sterile 0.22  $\mu\text{m}$  filter (Micro Filtration Systems, Dublin, CA), flushed with  $\text{N}_2$ , and incubated at  $37^{\circ}\text{C}$  in vials covered with Teflon-lined screw caps. The cholesterol crystal formation time represents the interval (in days) between time zero and the first appearance of plate-like cholesterol monohydrate crystals (50). The samples were observed for a maximum period of 21 days. When cholesterol crystals did not appear during the observation period, the nucleation time was recorded as 21 days.

### Statistical analysis

Data are presented as means  $\pm$  SD. Means were compared using Student's *t*-test or ANOVA. Chi-square tests were used to compare the frequencies of homozygous and heterozygous apoE genotypes (E2/E2, E2/E3, E2/E4, E3/E3, E3/E4, and E4/E4) in the different study groups. The sample size was estimated considering *i*) an apoE4 phenotype frequency of 20% in the general population with a sampling error of  $<5\%$  and *ii*) a statistical power of 80% to detect at least a 2-fold increase in the

TABLE 1. Clinical characteristics and plasma lipid and lipoprotein levels in Chilean subjects with and without gallstones

Characteristics and Levels	Patients with Gallstones (n = 117)	Patients without Gallstones (n = 122)	P
Age (years)	49 $\pm$ 12	40 $\pm$ 13	0.001
BMI ( $\text{kg}/\text{m}^2$ )	28.7 $\pm$ 5.0	26.4 $\pm$ 5.0	0.001
Total Chol (mg/dl)	198 $\pm$ 39	187 $\pm$ 39	0.02
HDL Chol (mg/dl)	47 $\pm$ 11	44 $\pm$ 11	0.04
LDL Chol (mg/dl)	124 $\pm$ 36	119 $\pm$ 3	NS
Triglycerides (mg/dl)	135 $\pm$ 86	120 $\pm$ 78	NS

BMI, body mass index; Chol, cholesterol.

TABLE 2. Clinical characteristics and plasma lipid and lipoprotein levels in German subjects with and without gallstones

Characteristics and Levels	Patients with Gallstones (n = 184)	Patients without Gallstones (n = 184)	P
Age (years)	63 $\pm$ 13	63 $\pm$ 13	NA
BMI ( $\text{kg}/\text{m}^2$ )	26.1 $\pm$ 4.3	25.4 $\pm$ 4.1	NA
Total Chol (mg/dl)	200 $\pm$ 53	202 $\pm$ 55	NS
HDL Chol (mg/dl)	48 $\pm$ 19	50 $\pm$ 20	NS
LDL Chol (mg/dl)	122 $\pm$ 46	125 $\pm$ 42	NS
Triglycerides (mg/dl)	143 $\pm$ 79	146 $\pm$ 105	NS

Chol, cholesterol; NA, not applicable (matched).

apoE4 frequency among gallstone patients compared with controls. Because in the Chilean study, subjects with GD were significantly older and heavier compared with controls and also differed in some metabolic variables (Table 1), we also analyzed genotypes grouped by whether or not they contained apoE4 by multivariate logistic regression analysis with adjustment for these confounding factors. Relative odds ratio estimates (with 95% confidence interval) of GD with the apoE genotype were determined after adjustment for age, gender, BMI, and serum lipids. The likelihood ratio statistic was used to test the significance of the regression models. The Kruskal-Wallis test was used to compare the cholesterol content of gallstones, cholesterol crystal formation time, and CSI in apoE genotype subgroups. Data storage and statistical analysis were performed using SAS/PC+ or SPSS 13.0.

## RESULTS

### Population characteristics

The random samples from Chilean and German adult populations included 239 and 368 individuals, respectively. **Tables 1, 2** summarize clinical data and lipid and lipoprotein levels in these study groups and in the subgroups of patients with and without gallstones. The Chilean sample population was markedly younger than the German subjects. As we observed in the whole Chilean survey (8), the selected Chilean GD subjects were slightly but significantly older, with higher BMI, total cholesterol, and fasting glycemia compared with subjects without GD. German subjects were deliberately matched for age and

TABLE 3. ApoE genotype and allele frequencies in Chilean subjects with and without gallstones

Genotype and Allele	All (n = 239)	Patients with Gallstones (n = 117)	Patients without Gallstones (n = 122)	P
ApoE genotype				
E2/E2	0.004	0.008	0.000	NS
E2/E3	0.067	0.051	0.082	NS
E2/E4	0.004	0.008	0.000	NS
E3/E3	0.73	0.79	0.67	NS
E3/E4	0.17	0.12	0.23	NS
E4/E4	0.017	0.017	0.016	NS
ApoE allele				
$\epsilon$ 2	0.07	0.07	0.08	NS
$\epsilon$ 3	0.73	0.79	0.67	NS
$\epsilon$ 4	0.19	0.13	0.24	NS

ApoE, apolipoprotein E.



TABLE 4. ApoE genotype and allele frequencies in German subjects with and without gallstones

Genotype and Allele	All (n = 368)	Patients with Gallstones (n = 184)	Patients without Gallstones (n = 184)	P
<b>ApoE genotype</b>				
E2/E2	0.000	0.000	0.000	NS
E2/E3	0.13	0.14	0.13	NS
E2/E4	0.024	0.027	0.022	NS
E3/E3	0.63	0.63	0.64	NS
E3/E4	0.20	0.20	0.20	NS
E4/E4	0.014	0.016	0.011	NS
<b>ApoE allele</b>				
ε2	0.08	0.08	0.08	NS
ε3	0.80	0.79	0.80	NS
ε4	0.12	0.13	0.12	NS

BMI, and both groups did not differ with respect to serum lipid levels.

**Distribution of apoE genotypes and allele frequencies in subjects with and without gallstones**

The observed distributions of apoE genotypes in both populations were in Hardy-Weinberg equilibrium (degrees of freedom = 5, all  $P > 0.05$ ). As expected, the ancestral wild-type apoE genotype E3/E3, carried by 63–73% of the subjects, was the most frequent in the populations we investigated. E3/E4 and E2/E3 had intermediate frequencies, and all other genotypes had very low frequencies, as shown in Tables 3, 4. These tables also summarize the distribution of apoE genotypes and allele frequencies between patients with and without gallstones. No significant differences of apoE genotypes and allele frequencies were detected. Accordingly, no odds ratio statistics were calculated for the matched German case-control population. In the Chilean population after adjusting for risk factors such as age, gender, and BMI, the odds ratio for the association between heterozygosity or homozygosity for the apoE4 allele and the prevalence of cholelithiasis was 0.39 (95% confidence interval, 0.18–0.83). Further adjustment for serum lipid and glucose levels did not substantially change these results (odds ratio, 0.40; 95% confidence interval, 0.18–0.86), suggesting that the apoE4 allele might even decrease the risk for gallstone development in this Hispanic population.

**ApoE genotypes and allele frequencies in gallstone-resistant individuals and gallstone-susceptible patients**

Table 5 summarizes the clinical characteristics of the gallstone-resistant and gallstone-susceptible subgroups

TABLE 5. Characteristics of gallstone-resistant and gallstone-susceptible patients within the Chilean population

Gallstone Phenotype	Age	Females	Pregnancies	BMI	Gallstones
	years	%	n	kg/m <sup>2</sup>	%
Resistant (n = 50)	58.3 ± 7.9	78	5.3 ± 2.6	31.9 ± 5.2	0
Susceptible (n = 32)	22.3 ± 7.8	94	0.4 ± 0.5	23.0 ± 2.7	100
P	<0.001	NS	<0.001	<0.001	<0.001

TABLE 6. ApoE genotypes and allele frequencies in gallstone-resistant individuals and gallstone-susceptible patients

Genotype and Allele	Gallstone-Resistant Patients (n = 50)	Gallstone-Susceptible Patients (n = 32)	P
<b>ApoE genotype</b>			
E2/E2	—	—	
E2/E3	2.0	3.1	NS
E2/E4	—	—	
E3/E3	78.0	87.5	NS
E3/E4	20.0	9.4	NS
E4/E4	—	—	
<b>ApoE allele</b>			
ε2	0.01	0.02	NS
ε3	0.89	0.94	NS
ε4	0.10	0.05	NS

of Chilean patients included in this study, and Table 6 shows the apoE genotype frequencies and allele frequencies of these two subgroups. Although there was even a tendency toward a higher frequency of E3/E4 in gallstone-resistant individuals compared with gallstone-susceptible patients, no significant differences between apoE genotype or allele frequencies were detected. The higher frequency of apoE3 alleles in this selected group of patients compared with the major Chilean cohort (Table 3) might be attributable to chance or could even indicate Amerindian heritage, because some Amerindian and Mexican-American cohorts display high apoE3 allele frequencies (52).

**Correlation between apoE polymorphisms and serum lipoprotein levels**

Tables 7, 8 summarize serum lipid levels in Chilean and German subjects with different apoE genotypes. We observed a significant association between apoE genotypes and total cholesterol as well as LDL cholesterol levels only in the German population. Patients carrying apoE4 genotypes displayed significantly higher total cholesterol and LDL cholesterol levels compared with patients without apoE4 (Table 8). A similar but not significant trend was observed in Chilean subjects (Table 7).

In regression analyses including age, gender, or BMI, LDL cholesterol levels increased significantly with BMI in the German cohort, and no significant relationships were observed for total cholesterol levels. In multivariate analysis after adjusting for BMI, apoE4 genotypes still contributed significantly ( $P < 0.01$ ) to higher LDL cholesterol levels. The slightly older age in German patients with apoE4 genotypes is attributed to chance, because the population frequencies of these genotypes are known to decrease with age (53).



TABLE 7. Plasma lipid and lipoprotein levels in Chilean subjects with distinct apoE genotypes

Level	E4/E4 (n = 4)	E4/non-E4 (n = 43)	non-E4/ non-E4 (n = 192)	P (ANOVA)
Age (years)	36 ± 17	42 ± 12	45 ± 13	NS
Total Chol (mg/dl)	204 ± 44	195 ± 41	192 ± 39	NS
HDL Chol (mg/dl)	40 ± 7	44 ± 10	45 ± 11	NS
LDL Chol (mg/dl)	144 ± 36	125 ± 34	120 ± 35	NS
Triglycerides (mg/dl)	96 ± 32	124 ± 69	128 ± 86	NS

Chol, cholesterol.

In the Chilean cohort, total and LDL cholesterol levels increased significantly with age ( $P < 0.001$ ) and BMI ( $P < 0.05$ ). HDL cholesterol levels were higher in women ( $P < 0.001$ ) and showed a negative correlation with BMI ( $P < 0.05$ ) and a positive correlation with age ( $P < 0.01$ ). Finally, triglyceride levels were higher in men ( $P < 0.01$ ) and increased with BMI ( $P < 0.001$ ). In multiple regression analysis after adjusting for age, gender, and BMI, apoE genotypes did not contribute to serum lipid levels (all  $P > 0.05$ ).

#### Correlation between apoE polymorphisms and bile lithogenicity

The group of cholecystectomized Chilean patients with cholesterol gallstones included 81 individuals (age,  $46 \pm 12$  years; 57% females; BMI,  $25 \pm 4$  kg/m<sup>2</sup>). The distribution of apoE genotypes in this group was 75.6% E3/E3, 18.1% E3/E4, 4.8% E2/E3, and 1.5% E4/E4, which was not significantly different from the genotypes detected in the total Chilean study cohort. To compare our findings with those of previous studies, we performed a correlation analysis between apoE genotypes and indexes of bile lithogenicity. **Table 9** summarizes the gallstone cholesterol content, cholesterol crystal formation time, and CSI of gallbladder biles of the apoE subgroups of patients with cholesterol gallstones. We did not observe any significant differences between these variables among the apoE subgroups.

### DISCUSSION

The hypothesis that apoE might be a genetic determinant of cholesterol GD is based on previous observations

TABLE 8. Plasma lipid and lipoprotein levels in German subjects with distinct apoE genotypes

Level	E4/E4 (n = 5)	E4/non-E4 (n = 81)	non-E4/ non-E4 (n = 282)	P (ANOVA)
Age (years)	69 ± 10	60 ± 14	64 ± 13	<0.05
Total Chol (mg/dl)	245 ± 55	212 ± 58	197 ± 52	<0.05
HDL Chol (mg/dl)	65 ± 22	49 ± 18	49 ± 20	NS
LDL Chol (mg/dl)	154 ± 40	135 ± 47	119 ± 42	<0.01
Triglycerides (mg/dl)	157 ± 68	146 ± 87	144 ± 95	NS

Chol, cholesterol.

indicating that cholesterol metabolism differs among individuals with distinct apoE isoforms. An association between apoE and cholesterol GD was suggested for the first time by a study performed in Finland (28), which reported an association of apoE4 (E4/E4 and E4/E3 genotypes) with increased cholesterol contents of gallstones and shorter cholesterol crystal formation times in gallbladder bile. However, apoE4 frequencies were similar between gallstone patients and control subjects. A second case-control study performed in Spain (29) indicated that the apoE4 isoform occurs more frequently in patients with gallstones compared with stone-free subjects, but these differences were significant only in women. In contrast to the Finnish study, other authors did not detect an association of apoE4 with an increased cholesterol content of gallstones or rapid cholesterol crystal formation (29, 34, 35). On the other hand, higher stone recurrence rates after extracorporeal shock-wave lithotripsy has been described in apoE4 carriers (54). In addition, upon challenge with a high-cholesterol diet, *ApoE* knockout mice showed a markedly lower frequency of gallstones than wild-type controls (55). Accordingly, apoE has been considered a candidate gene that may influence the susceptibility to cholesterol gallstone formation (4). However, it is unknown whether the genetic association can be replicated in larger, clinically relevant populations or populations with different ethnic backgrounds.

Chile has one of the highest gallstone prevalence rates (31% and 17% in adult women and men, respectively) worldwide (8). A high prevalence of GD has also been observed in Mexican Americans (56, 57) and Pima Indians, suggesting that the susceptibility to GD might be modulated by genetic components related to the degree of Amerindian admixture (58–60). Cholelithiasis is also a very common disease in Germany, with the latest population-based survey showing a standardized global gallstone prevalence of 19% (41). However, in both populations, we found no significant differences between the distribution of apoE genotypes in patients with gallstones compared with individuals without gallstones. The logistic regression analysis suggested that the apoE4 allele might be associated with a decreased frequency of GD in the Chilean case-control study. Furthermore, the lack of association between apoE and GD in our populations was confirmed when we compared gallstone-susceptible and gallstone-resistant patients, an approach that can increase the chance of detecting the contribution of genetic factors involved in GD, because such as genetic study design maximizes statistical power (61, 62).

Our results contrast with the increased risk of GD in apoE4 carriers reported in Finland (28, 30) and Spain (29). How can we explain these apparently contradictory findings? One possibility is the presence of a selection bias in previous case-control studies. In fact, the only study that shows a positive association between apoE4 and GD per se (29) had an unusually low apoE4 allele frequency (4%) in the control group, which differs from the frequency described for the general Spanish population (63, 64). Different ethnicities with lower gallstone prevalence and the

TABLE 9. Cholesterol content of gallstones, cholesterol crystal formation time, and CSI in apoE subgroups of Chilean patients with cholesterol gallstones (n = 81)

Variable	ApoE Isoforms <sup>a</sup>			P
	E2 (n = 4)	E3 (n = 61)	E4 (n = 16)	
Gallstone cholesterol content (%)	67.0 ± 19.2	67.9 ± 14.4	65.6 ± 12.5	NS
Cholesterol crystal formation time (days)	7.3 ± 9.2	7.4 ± 7.3	6.0 ± 6.7	NS
CSI	119.4 ± 34.4	128.2 ± 41.5	101.9 ± 27.5	NS

CSI, cholesterol saturation index.

<sup>a</sup>ApoE isoform E2 = apoE genotypes E2/E2, E2/E3, and E2/E4; apoE isoform E3 = apoE genotype E3/E3; apoE isoform E4 = apoE genotypes E4/E4 and E4/E3.

inclusion of younger control probands might contribute to these discrepancies, because apoE4 allele frequencies are low in Asian populations (31, 32, 63) and decrease with age as a result of increased serum LDL cholesterol levels and increased risk for cardiovascular mortality (26). In our study, the apoE allele distributions in Chile and Germany were in accordance with the distributions expected for populations with Spanish-Amerindian admixture or primary Caucasian origin, respectively (63): intermediate between the Asian studies (31–33) and the higher apoE4 frequencies (28–33%) in the Finnish studies (28, 30). Furthermore, in agreement with other studies (65), apoE4 genotypes of German patients were associated with higher LDL cholesterol levels in serum, whereas no such association was detected in the Chilean population. The latter finding parallels observations in Japanese and Singaporean populations (66), and it is well established that populations with Amerindian background are closer to Asian than to Caucasian populations.

Because >95% of gallstones from Chilean patients are composed mainly of cholesterol, it is very likely that apoE polymorphism is not associated with cholesterol GD in this population. Accordingly, there was no correlation between apoE genotypes and biliary cholesterol saturation. Specifically, apoE4 was not associated with higher cholesterol content of gallstones, higher CSI, or shorter biliary cholesterol crystal formation time. Of note, in the study by Juvonen et al. (28), many patients had pigment gallstones and stone cholesterol contents were higher and cholesterol nucleation times were shorter in subjects with apoE4 alleles, suggesting that apoE genotypes might determine whether patients form cholesterol or pigment stones. Because virtually all Chilean patients have cholesterol gallstones and stone type was not determined in the German population reported here, the current study cannot exclude the possibility that apoE4 status determines stone type. In fact, a single study has reported higher ε4 allele frequencies in patients with cholesterol stones compared with patients with pigment stones (67).

In summary, our findings indicate that apoE variants do not play a significant role in modulating the predisposition to cholesterol gallstone formation in Chileans and Germans, two populations with very high frequencies of GD. Screening for carriers of common apoE4 variants cannot be recommended on the basis of the current association studies and does not guide prevention or therapy for cholesterol gallbladder stones. ■

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