PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

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Effect of apolipoprotein E gene Hha I restricting fragment length polymorphism on serum lipids in cholecystolithiasis

LIN Qi-Yuan, DU Jing-Ping, ZHANG Ming-Yi, YAO Yu-Gwei, Li Lin, CHENG Nan-Sheng,YAN Lu-Nan and XIAO Lu-Jia

Subject headings apolipoprotein E; polymorphism; lipids; cholecystolithiasis; polymerase chain reaction

Abstract

AIM **To investigate the role of apolipoprotein E (apoE) polymorphism in the lithogenesis of gallstone and the hereditary pathogenesis of the disease.**

METHODS **Polymerase chain reaction (PCR) was used to study apoE phenotypes and allele frequencies in patients with gallstones and control, and the fasting serum lipids of subjects were also measured by enzymatic methods.**

RESULTS **The levels of triglyceride (TG) and very low density lipoprotein cholesterol (VLDL-C) were much higher in E**2/3**patients than that in E**2/3 **control. E**3/3 **patients were accompanied with remarkably low levels of high density lipoprotein cholesterol(HDL-C) and its subforms. But in E**3/4 **patients there were only slight changes in levels of VLDL-C and low density lipoprotein cholesterol (LDL-C).**

CONCLUSION **Different apoE phenotype patients with gallstones have different characteristics of dyslipidemia and the average level of serum lipids in patients with gallstones are higher than subjects without gallstones in the same apoE gene phenotype.** ε2 **allele is possibly one of the dangerous factors in the lithogenesis of cholecystolithiasis.**

Department of General Surgery, First Hospital, West China University of Medical Sciences

Dr. LIN Qi-Yuan, Male, born on 1964-01-01 in Nanchong City, Sichuan Province, graduated from West China University of Medical Sciences and earned a Doctor degree in 1996, now an associate professor, majoring hepatic-biliary-pancreatic surgery having 16 articles published.

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Correspondence to: LIN Qi-Yuan, Department of General Surgery, First University Hospital, West China University of Medical Sciences, Chengdu 610041, Sichuan Province, China
Tel. +86 • 28 • 5551078, Fax. +86 • 28 • 5530724
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INTRODUCTION

The apolipoprotein E (apoE) gene locus possesses three alleles, ε2, ε3 and ε4, which are inherited in co-domain fashion and code for three isoprotein E2, E3 and E4 making up six phenotypes, three heterozygous $E_{2/3}$, $E_{3/4}$ and $E_{2/4}$, three homozygous $E_{2/2}$, $E_{3/3}$ and $E_{4/4}$[1-7]. The differences of these main isoprotein alter the receptor-binding affinity of the apolipoprotein-containing lipoproteins and affect the metabolism of cholesterol and lipids[2,4]. It is putative that apoE polymorphisms are closely related to hyperlipidemia[5], coronary heart disease [6] and diabetes mellitum[7]. The formation of gallstones is frequently associated with the changes in biliary lipid compositions, the lithogenic bile being usually supersaturated with cholesterol and decreased with bile acids and lecithin[8,9]. A prerequisite for the formation of gallstones is the lithogenic bile, which is often the result of disorders in lipid metabolism or dyslipidemia. The important role of apoE in the regulation of lipid metabolism raises the possibility that apoE polymorphisms may be involved in the formation of gallstones. This case-control cohort study is designed to investigate the significance of apoE polymorphisms as a predisposing factor in the pathogenesis of cholecystolithiasis.

SUBJECTS AND METHODS

Subjects

Eighty-seven consecutive patients with gallstones were investigated. The treatment group consisted of 39 men and 48 women (mean age 52 years, ranging from 16 to 83 years). All of them suffered from non-symptomatic cholecystolithiasis and underwent operation in the First Hospital from January 1994 to December 1995. The control group included 50 subjects with 27 men and 23 women (mean age 49 years, ranging from 15 to 78 years), and they were also matched in sex and age distribution with the patients with gallstones.

DNA amplification

Leukocyte DNA of venous blood collected in EDTA tubes were extracted by Hixson slotting-out method [10]. Model DNA was amplified by polymerase chain reaction (PCR) thermal cycles using oligonucleotides primers F4 (5'- ACAGAATTCGCC CCGGCCTGGTACAC-3') and F6 (5'-TAAGCTTGG CACGGCTG TCCAAGGA-3'). Each amplification reaction system contained 1 μg DNA, 1pmol/L

of each primer and 25 kilo units/L of Taq-polymerase up to a final volume of 30 μL. Each reaction mixture was heated at 95℃ for 5 minutes for predenaturation, and followed by 30 cycles of amplification for annealing at 60℃ for 1 minute, elongation at 70℃ for 2 minutes, denaturation at 95℃ for 1 minute, and then a prolonged elongation time up to 7 minutes at 56℃.

Analysis of restricting fragment length polymorphism for apoE

Twenty-five μL of PCR amplified products in each react ion system were mixed with 5 units of Hha I enzyme for digestion apoE sequences at 37℃ for 1 hour. Each reaction mixture was loaded onto 85 g/L polyacrylamine gel, after electrophoresis for 3 hours under constant current (45 mA) and visualized by ultraviolet light. The size of apoE Hha I restricting fragment length polymorphsims were estimated by comparison with marker DNA PBR32. On the basis of the size and the number of various fragments, apoE phenotypes were determined as E2 with 91bp , and 83bp E3 with 91bp, 48bp and 35bp, as well as E4 with 72bp, 48bp and 35bp.

Lipids analysis

Serum total cholesterol (TC) and total triglyceride (TG) were determined by enzy matic methods with the OUL 3 000 automatic analyzer. High density lipoprote in cholesterol (HDL-C) was measured enzymatically and formed in the serum supernatant after precipitation of low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) with dextrin sulfate and MgCl2. The LDL-C and VLDL-C levels were calculated according to Friedwald's formula[11].

Statistical analysis

All results were expression as $x \pm s$. The F test and χ^2 test were used for statistical analysis, P values less than 0.05 were regarded as significant.

RESULTS

Distribution of apoE phenotypes and allele frequencies

In the six common apoE phenotypes, $E_{2/3}$, $E_{3/3}$ and $E_{3/4}$ phenotypes existed in either patients with gallstones or control subjects. There were only 2 $E_{2/4}$ phenotype cases in the control, and no $E_{2/2}$ and $E_{4/4}$ were detected in both groups. The overall distribution of apoE phenotypes and apoE allele frequencies in the patients with gallstones were analogous to that of the control (Table 1).

Table 1 Apolipoprotein E phenotype distributions and allele frequenc ies in patients with gallstones and controls

Groups	*n*	Phenotype				Allele		
		$E_{2/3}$(%)	$E_{2/4}$(%)	$E_{3/3}$(%)	$E_{3/4}$(%)	ε2(%)	ε3(%)	ε4(%)
Patients	87	10(11.4)	0	69(79.4)	8(9.2)	5.8	89.6	4.6
Male	39	4(10.2)	0	31(79.4)	4(10.2)	5.1	89.8	5.1
Female	48	6(12.5)	0	38(79.1)	4(8.9)	6.3	89.6	4.2
Controls	50	5(10.0)	2(4.0)	37(74.1)	6(12.0)	7.0	85.0	8.0
Male	27	3(10.0)	1(3.7)	20(74.0)	3(11.0)	7.4	85.2	7.4
Female	23	2(8.6)	1(4.3)	17(74.6)	3(13.0)	6.5	84.8	8.7

Serum lipids

The levels of TG (1.43 mmol/L) and VLDL-C (0.68 mmol/L) in $E_{2/3}$ patients with gallstones were markedly higher than that in $E_{2/3}$ control (1.06 mmol/L, P<0.05 and 0.48 mmol/L, P<0.05). LD L-C (1.41 mmol/L was significantly lower in $E_{2/3}$ patients than that in the control (2.04 mmol/L , P<0.05). No statistical differences were noted in TC, HDL-C, HDL2-C and HDL3-C between $E_{2/3}$ patients and control subjects (Table 2).

In $E_{3/3}$ patients with gallstones, the HDL-C (0.89 mmol/L), HDL2-C (0.49 mmol/L) and HDL3-C (0. 39 mmol/L) were significantly decreased as compared with that in $E_{3/3}$ control (1.28 mmol/L, P <0.0 5; 0.73 mmol/L P<0.001; and 0.55 mmol/L, P <0.001). LDL-C and VLDL-C showed no difference in both groups (Table 2). $E_{3/3}$ female patients had lower levels of HDL-C (0.82 mmol/ L), HDL2-C (0.46 mmol/L) and HDL3-C (0.36 mmol/L) than $E_{3/3}$ female controls (1.33 mmol/L, P <0.001; 0.77 mmol/L, P<0.01; and 0.57 mmol/L, P <0.01). Serum lipid levels were not changed in $E_{3/3}$ male patients and controls (Table 3).

LDL-C increased (1.92 mmol/L) and VLDL-C decreased (0.42 mmol/L) in $E_{3/4}$ patients with gallstones as compared with $E_{2/3}$ patients (LDL-C-1.41 mmol/L, VLDL-C-0.68 mmol/L) and $E_{3/3}$ patients (LDL-C 1.87 mmol/L, VLDL-C 0.46 mmol/L), but the differences were not significant. No obvious changes occurred in TC or HDL-C and its subforms among $E_{2/3}$, $E_{3/3}$ and $E_{3/4}$ patients with gallstones (Table 2).

Table 2 Comparisons of lipid levels in $E_{2/2}$, $E_{3/3}$, $E_{3/4}$ both gallstone patients and controls

Lipids (mmol/L)	$E_{2/3}$		$E_{3/3}$		$E_{3/4}$	
	Patients (n = 10)	Controls (n = 5)	Patients (n = 69)	Controls (n = 37)	Patients (n = 8)	Controls (n = 6)
TG	1.43 ± 0.35[a]	1.06 ± 0.10	0.97 ± 0.21	0.64 ± 0.44	1.11 ± 0.33	0.92 ± 0.16
TC	2.99 ± 0.65	2.52 ± 0.53	3.14 ± 0.59	3.67 ± 0.76	3.94 ± 0.45	3.62 ± 0.63
LDL-C	1.41 ± 0.56[a]	2.04 ± 0.16	1.87 ± 0.49	2.43 ± 0.67	1.92 ± 0.64	2.46 ± 0.32
VLDL-C	0.68 ± 0.26[a]	0.48 ± 0.20	0.46 ± 0.20	0.30 ± 0.11	0.42 ± 0.13	0.44 ± 0.10
HDL-C	0.95 ± 0.23	1.02 ± 0.15	0.89 ± 0.30[a]	1.28 ± 0.23	0.86 ± 0.21	0.90 ± 0.36
HDL2-C	0.53 ± 0.13	0.62 ± 0.22	0.49 ± 0.18[b]	0.73 ± 0.13	0.44 ± 0.19	0.55 ± 0.18
HDL3-C	0.42 ± 0.12	0.56 ± 0.28	0.39 ± 0.12[b]	0.55 ± 0.11	0.40 ± 0.13	0.46 ± 0.12

[a]P<0.05, [b]P<0.01, *vs* controls; F test.

Table 3 The comparisons of lipid levels in $E_{3/3}$ same gender ei ther gallstone patients or controls

Lipids (mmol/L)	Male		Female	
	Patients (n = 31)	Controls (n = 20)	Patients (n = 38)	Controls (n = 17)
TG	0.85 ± 0.50	0.53 ± 0.22	1.10 ± 0.30	0.72 ± 0.57
TC	3.05 ± 0.44	0.41 ± 0.57	3.23 ± 0.85	3.87 ± 0.63
LDL-C	1.86 ± 0.68	2.71 ± 0.49	1.89 ± 0.86	2.20 ± 0.56
VLDL-C	0.40 ± 0.24	0.25 ± 0.10	0.52 ± 0.14	0.35 ± 0.17
HDL-C	0.94 ± 0.33	1.21 ± 0.28	0.82 ± 0.27[b]	1.33 ± 0.19
HDL2-C	0.52 ± 0.22	0.69 ± 0.16	0.46 ± 0.15[b]	0.77 ± 0.10
HDL3-C	0.42 ± 0.12	0.52 ± 0.13	0.36 ± 0.12[b]	0.57 ± 0.10

[b]P<0.01, *vs* controls, F test.

DISCUSSION

$E_{2/3}$, $E_{3/3}$, and $E_{3/4}$ are three common apolipoprotein E gene phenotypes, accounting for more than 50%, $E_{2/4}$ and $E_{4/4}$ for less than 6.2%[12]. In the present study, only 2 $E_{2/4}$ phenotype cases were detected in control, and no $E_{2/2}$ and $E_{4/4}$ homozygotes were found in both groups. The results show that ε2 and ε4 alleles resulting from the inheridary variations of apoE gene existed mainly in heterozygous way in population.

There were racial differences in the distribution of apoE alleles and phenotypes. In this study and Wang's literature[13], the frequencies of $E_{3/3}$ phenotype were 85%-86% in healthy Chinese people, but 75% in Finnish people. Frequencies of ε4 were lower in Chinese people (8%-9%) than 20% in the Finnish (20%). The frequencies of $E_{3/3}$ phenotype in Chinese patients with gallstones were 79.3% as compared with 62.2% in Finnish, and $E_{3/4}$ phenotype in Chinese patients with gallstones were 9.2% but 28.9% in Finnish[14]. Kamb oth[15] also reported that there may be some variations of apoE allele and phenotype in different regional population from western to oriental countries .

Patients of different apoE phenotype with gallstones had different characteristics of dyslipidemia. Higher mean serum TG, VLDL-C levels and lower mean LDL-C levels were found in $E_{2/3}$ patients. The $E_{3/3}$ patients, especially in women, had markedly lower concentrations of HDL-C, HDL2-C and HDL-3-C, while $E_{3/4}$ patients had only slight lower levels of VLDL-C and higher levels of LDL-C as compared with the $E_{2/3}$ and $E_{3/3}$ patients with gallstones.

The difference in the changes of serum lipid levels in different apoE phenotype patients with gallstone may be associated with apoE locus gene polymorphisms. E2, E3 and E4 isoproteins resulted from the single amino acid interchange between 112 site cysteine and 118 site arginine, E3 with cysteine at 112 site and arginin at 18 site, E2 with cysteine and E4 with arginine at either 112 site or 118 sites. Because of arginine bearing positive charge, E4 possessed more than one charge, the activity of receptor-binding to apoE-contained lipoprotein was strong er than E3. On the contrary, E2 possessed less than one charge, the activity of receptor-binding was lower[1-3]. Accordingly, ε2 allele predisposes to serum triglyceride elevation[7], the correlative change to serum lipid levels can be found in $E_{2/3}$ patients with gallstones in this study. ε4 allele was responsible for the increase of serum cholesterol[16], but in $E_{3/4}$ phenotype patients, the increments of VLDL-C had no statistical difference, this may be associated with the low frequency of ε4 allele in population.

$E_{3/3}$ phenotype is putative normal type, but the $E_{3/3}$ patients with gallstones possessed the low level of HDL-C and its subforms as well. The ch anges may be related to other pathogenesis except apoE polymorphisms[17]. The results suggest that cholecystolithiasis may be a multigenic disease but not a monogenic one.

This study demonstrates that patients of different apoE phenotype with gallstones possess different dyslipidemia. The average level of serum lipids are much higher in patients with gallstones than that in non-gallstone subjects in the same apoE phenotype population. ε2 allele is likely one of the high-rish factors in the lithogenesis of cholecystolithiasis.

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