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

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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 E2/3 patients than that in E2/3 control. E3/3 patients were accompanied with remarkably low levels of high density lipoprotein cholesterol (HDL-C) and its subforms. But in E3/4 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.

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 E2ε3, E3ε4 and E2ε4, three homozygous E2ε2, E3ε3 and E4ε4[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[2-9]. It is putative that apoE polymorphisms are closely related to hyperlipidemia[10], 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'-ACAGAA TTCGCC CCGCCTGGTACAC-3') and F6 (5'-TAAGCTTGG CACGCGCTG TCCAAGGA-3'). Each amplification reaction system contained 1 μg DNA, 1 pmol/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 °C for 5 minutes for pre-denaturation, and followed by 30 cycles of amplification for annealing at 60 °C for 1 minute, elongation at 70 °C for 2 minutes, denaturation at 95 °C for 1 minute, and then a prolonged elongation time up to 7 minutes at 56 °C.

Analysis of restricting fragment length polymorphism for apoE
Twenty-five µL of PCR amplified products in each reaction system were mixed with 5 units of Hha I enzyme for digestion apoE sequences at 37 °C for 1 hour. Each reaction mixture was loaded onto 85 g/L polyacrylamide 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 polymorphisms 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 enzymatic methods with the OUL 3000 automatic analyzer. High density lipoprotein 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 HDL-C and VLDL-C levels were calculated according to Friedwald’s formula[11].

Statistical analysis
All results were expression as x ± s. The F test and χ² 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, E2/3, E3/3 and E3/4 phenotypes existed in either patients with gallstones or control subjects. There were only 2 E2/4 phenotype cases in the control, and no E2/2 and E4/4 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 2 Comparisons of lipid levels in E2/2, E2/3, E2/4 both gallstone patients and controls

| Lipids | E2/2 | E2/3 | E2/4 |
|--------|------|------|------|
| Patients | Controls | Patients | Controls | Patients | Controls |
| TG | 1.43 ± 0.35a | 1.06 ± 0.10a | 0.97 ± 0.21 | 0.64 ± 0.44 | 1.11 ± 0.33b | 0.92 ± 0.16 |
| TC | 2.99 ± 0.65a | 2.52 ± 0.53 | 3.14 ± 0.59 | 3.67 ± 0.76 | 3.94 ± 0.45b | 3.62 ± 0.63 |
| LDL-C | 1.41 ± 0.56a | 2.04 ± 0.16 | 1.87 ± 0.49 | 2.43 ± 0.67 | 1.92 ± 0.64 | 2.46 ± 0.32 |
| VLDL-C | 0.68 ± 0.26a | 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 | 1.28 ± 0.23 | 0.86 ± 0.21 | 0.90 ± 0.36 |
| HDL2-C | 0.53 ± 0.13 | 0.62 ± 0.22 | 0.49 ± 0.18b | 0.73 ± 0.13 | 0.44 ± 0.19 | 0.55 ± 0.18 |
| HDL3-C | 0.42 ± 0.12 | 0.56 ± 0.28 | 0.39 ± 0.12b | 0.55 ± 0.11 | 0.40 ± 0.13 | 0.46 ± 0.12 |

⁎P<0.05, ⁎⁎P<0.01, vs controls; F test.

Serum lipids
The levels of TG (1.43 mmol/L) and VLDL-C (0.68 mmol/L) in E2/3 patients with gallstones were markedly higher than that in E2/3 control (1.06 mmol/L, P<0.05 and 0.48 mmol/L, P<0.05). LDL-C (1.41 mmol/L) was significantly lower in E2/3 patients than 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 E2/3 patients and control subjects (Table 2).

In E3/3 patients with gallstones, the HDL-C (0.89 mmol/L), LDL-C (0.49 mmol/L) and HDL-C (0.39 mmol/L) were significantly decreased as compared with that in E3/3 control (1.28 mmol/L, P<0.05; 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). E3/3 female patients had lower levels of HDL-C (0.82 mmol/L), LDL-C (0.46 mmol/L) and HDL-C (0.36 mmol/L) than E3/3 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 E3/3 male patients and controls (Table 3).

LDL-C increased (1.92 mmol/L) and VLDL-C decreased (0.42 mmol/L) in E3/3 patients with gallstones as compared with E3/3 patients (LDL-C:1.41 mmol/L, VLDL-C:0.68 mmol/L) and E3/3 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 E2/3, E3/3, and E3/4 patients with gallstones (Table 2).
E2/4 phenotype cases were detected in controls, and no C and higher levels of LDL-C as compared with the E2/3 and E3/3, and E3/4 are three common apolipoprotein E gene phenotypes, accounting for more than 50%, E2/4 and E4/4 for less than 6.2%[12]. In the present study, only 2 E2/4 phenotype cases were detected in control, and no E2/2 and E4/4 homozygotes were found in both groups. The results show that e2 and e4 alleles resulting from the inhereditary 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 E3/3 phenotype were 85%-86% in healthy Chinese people, but 75% in Finnish people. Frequencies of e4 were lower in Chinese people (8%-9%) than 20% in the Finnish (20%). The frequencies of E3/3 phenotype in Chinese patients with gallstones were 79.3% as compared with 62.2% in Finnish, and E3/4 phenotype in Chinese patients with gallstones were 9.2% but 28.9% in Finnish[14]. Kamb et al[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 HDL-C, HDL2-C and HDL-3-C, patients, especially in women, had markedly lower concentrations of HDL-C, HDL2-C and HDL-3-C, while E3/4 patients had only slight lower levels of VLDL-C and higher levels of LDL-C as compared with the E2/3 and E3/3 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 and 118 site. Because of arginine bearing positive charge, E4 possessed more than one charge, the activity of receptor-binding to apoE-contained lipoprotein was stronger than E3. On the contrary, E2 possessed less than one charge, the activity of receptor-binding was lower[1-3]. Accordingly, e2 allele predisposes to serum triglyceride elevation[7], the correlative change to serum lipid levels can be found in E2/3 patients with gallstones in this study. e4 allele was responsible for the increase of serum cholesterol[16], but in E3/4 phenotype patients, the increments of VLDL-C had no statistical difference, this may be associated with the low frequency of e4 allele in population. E2/4 phenotype is putative normal type, but the E3/3 patients with gallstones possessed the low level of HDL-C and its subforms as well. The changes may be related to other pathogenesis except apoE polymorphisms[17]. The results suggest that cholelithiasis 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. e2 allele is likely one of the high-risk factors in the lithogenesis of cholelithiasis.

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**Table 3 The comparisons of lipid levels in E3/3 same gender either 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 ± 0.37 | 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 | 0.32 ± 0.14 | 0.35 ± 0.17 |
| HDL-C    | 0.94 ± 0.33 | 1.31 ± 0.28 | 0.82 ± 0.27 | 1.33 ± 0.19 |
| HDL2-C   | 0.52 ± 0.22 | 0.69 ± 0.16 | 0.46 ± 0.15 | 0.77 ± 0.10 |
| HDL3-C   | 0.42 ± 0.12 | 0.52 ± 0.13 | 0.36 ± 0.12 | 0.57 ± 0.10 |

*P<0.01, vs controls, F test.