Association of apolipoprotein A1-C3 gene cluster polymorphisms with gallstone disease

Manjusha Dixit PhD, Gourdas Choudhuri MD DM, Rajan Saxena MS, Balraj Mittal PhD

INTRODUCTION: Genetic polymorphisms in apolipoprotein genes may be associated with alteration in lipid profile and susceptibility to gallstone disease.

AIM: To determine the association between apolipoprotein A1 (APOA1) (–75 guanine [G] to adenine [A] and +83/84 M2+/–, MspI) and apolipoprotein C3 (APOC3) (SstI) polymorphisms with gallstone disease.

METHODS: MspI polymorphisms of the APOA1 gene and SstI polymorphisms of APOC3 were analyzed in DNA samples of 214 gallstone patients and 322 age- and sex-matched healthy controls. All statistical analyses were performed using SPSS version 11.5 (SPSS, USA) and Arlequin version 2.0 (Arlequin, Switzerland).

RESULTS: The APOA1 –75 G/A polymorphism was significantly associated with gallstone disease. Patients with the GG genotype (P=0.015) and G allele carriers (P=0.004) had a significantly higher risk of gallstone disease (1.087-fold and 1.561-fold, respectively), whereas patients with AA genotypes (P=0.011) and A allele carriers (P=0.004) were protected against gallstone disease. APOA1 +83 M2+/– and APOC3 SstI polymorphisms were not associated with gallstone disease. Case-control analysis of haplotypes showed a significant association in males only. G-M2+–S1 conferred risk for gallstone disease (P=0.036; OR 1.593, 95% CI 1.029 to 2.464), while A-M2+–S1 was protective (P=0.002; OR 0.370, 95% CI 0.197 to 0.695) against gallstone disease. In APOA1–75, APOA1+83 bilocus haplotypes, G-M2+ was associated (P=0.0001) with very high risk (OR 3.173, 95% CI 1.774 to 5.674) for gallstone disease in males only. APOA1–75,APOC3SstI haplotypes also showed significant association while APOA1+83,APOC3SstI haplotypes showed no association with gallstone disease.

CONCLUSIONS: The APOA1–75 G/A polymorphism is associated with gallstone disease and shows sex-specific differences. On the other hand, APOA1 M2+– and APOC3 SstI polymorphisms may not be associated with gallstone disease. Haplotype analysis is a better predictor of risk for gallstone disease.

Key Words: APOA1-C3 haplotype; APOA1 MspI polymorphism; APOC3 SstI polymorphism; Gallstone disease; Gene polymorphisms

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Gallstone disease is one of the common causes of abdominal pain, inflammation and infection of the gallbladder and the pancreas. However, long-standing gallstones have also been attributed to carcinoma of the gallbladder (1-3). Extensive data over the past 50 years have shown that gallstones result from complex interactions between genetic and environmental factors.

Environmental factors contribute significantly to gallstone disease. High concordance of cholecystitis in monozygotic twins (4,5) and clustering of cases in families in whom gallstone disease is diagnosed in childhood (6) have provided evidence for a genetic basis of the disease.

The pathophysiology of gallstone formation is complex. A number of epidemiological surveys have shown an association between altered plasma lipid levels and gallstone disease, especially decreased levels of high-density lipoprotein (HDL) cholesterol (7) and increased levels of both low-density lipoprotein cholesterol (8) and triglycerides (9). Plasma lipid and lipoprotein metabolism is controlled by activities of various enzymes and apolipoproteins (APO), which are the structural components of the lipoproteins (10). One candidate locus that has produced inconsistent linkage and association results for dyslipidemias is the APOA1-C3-A4-A5 gene complex, located on 11q23 (11). APOA1 constitutes a key component of the reverse cholesterol transport process (12). Furthermore, it is an activator of lecithin cholesterol acyltransferase, an enzyme that catalyzes the esterification of cholesterol in plasma (13).

A common variant due to guanine (G) to adenine (A) transition (G/A) has been described 75 base pairs upstream (~75 bp) from the APOA1 gene transcription start site. Several studies have reported that individuals with the A allele, which occurs at a frequency of 0.15 to 0.20 in Caucasian populations, have higher levels of HDL cholesterol and/or APOA1 than individuals that are homozygous for the most common G allele (14,15). Another polymorphism (M2+/–) is present at the +83/84 bp site in the first intron of the APOA1 gene, which is created by a cytosine (C) to thymine (T) (+83 bp) and/or a G/A (+84 bp) transition. Some studies have been performed on the association of the M2+/– polymorphism with lipid traits but the results have not been consistent (16-21).

APOC3 is a major constituent of chylomicrons and very low-density lipoprotein particles. Several DNA polymorphisms have been reported in the APOC3 promoter region (22). These mutations are in linkage disequilibrium with an SstI polymorphism in the 3' untranslated region (C3238G) (23).

Polymorphisms of these genes may alter the lipid levels in individuals, which may predispose a person to gallstone disease. If alleles that predispose individuals to lipid alterations can be identified, screening for the presence of these alleles may identify a substantial proportion of high-risk individuals. Appropriate monitoring of these individuals, in conjunction with targeted intervention, could then influence the onset of disease.

The present study was undertaken to determine the association between APOA1 MspI polymorphisms and APOC3 SstI polymorphisms with gallstone disease.

**METHODS**

**Subjects**
The present study comprised 214 gallstone patients (mean age 44.7±13.20 years) and 322 controls (mean age 43.98±11.46 years). All subjects were from north India. The gallstone patients were recruited among inpatients undergoing cholecystectomy and outpatients attending the clinics of the Department of Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences in Lucknow, India. Initially, 350 controls were recruited from the healthy staff members of the institute and the general population of the region. Three hundred twenty-two age- and sex-matched subjects who were found to be negative for gallstone disease (by ultrasound), diabetes mellitus, obesity and other chronic debilitating diseases were included in the study. The study was approved by the local ethical committee of the institute. After informed consent was given, blood was taken in EDTA for analysis of DNA. The genomic DNA was extracted from peripheral blood leukocyte pellets using the standard salting-out method (24).

**Genotyping**

APOAI and APOC3 gene fragments that encompassed polymorphisms were amplified by polymerase chain reaction (PCR) in a DNA thermal cycler (DNA Engine PTC-100, MJ Research, Inc, USA). The APOAI gene for the MspI polymorphism was amplified using the following primers: forward 5’-TTC CAT GGT TCC CTA CAG AGG AGT-3' and reverse 5’-TTA GGG GAC ACC TAC CCG CCA GGA GCA GCA AGC TGT-3' (26). Each amplification was performed using 100 ng to 300 ng of genomic DNA in a volume of 25 μL using 12.5 pmol of each primer, 200 μM dNTP, 15 mM magnesium chloride, 100 mM Tris (pH 8.0) and two units of Taq polymerase (Fermantas Inc, USA). DNA templates were initially denatured at 95°C for 3 min; for APOAI MspI, that procedure was followed by 30 cycles with denaturation at 95°C for 30 s, annealing at 60°C for 45 s, extension at 72°C for 60 s and final extension at 72°C for 5 min. The 435 bp PCR product was digested with five units of restriction enzyme MspI (Fermantas Inc) for 3 h at 37°C. The digested product was run on 5% polyacrylamide gel. Two sites located at ~75 bp and +83/84 bp were present in the amplified fragment. Based on restriction pattern, allelic pattern (M2+, 209 bp and 46 bp; G, 114 bp and 66 bp) was determined.

For the APOC3 SstI polymorphism, genomic DNA was initially denatured at 95°C for 5 min, and cycling conditions included denaturation at 95°C for 60 s, annealing at 59.5°C for 60 s, extension at 72°C for 60 s for 30 cycles and, finally, an extension at 72°C for 5 min. PCR product was digested with 5 U of SstI for a period of 3 h to 5 h and was run on 1.5% agarose gel for genotyping. The S1 allele gives a 596 bp band and the S2 allele gives two bands of 371 bp and 225 bp.

**Statistical evaluation**

To examine whether the genotype frequencies were in Hardy-Weinberg equilibrium, the \(\chi^2\) goodness of fit test was used. Haplotype frequencies were determined by the maximum likelihood method, using the expectation maximization algorithm. Pair-wise linkage disequilibrium between each pair of APOAI-C3 loci was analyzed using a likelihood ratio test, whose empirical distribution was obtained by a permutation procedure. The above calculations were performed using Arlequin version 2.0 software (Arlequin, Switzerland).
RESULTS

Table 1 shows age, male to female ratio and BMI in gallstone patients and controls. Differences in age, male to female ratio and BMI were insignificant.

All genotype distributions were in Hardy-Weinberg equilibrium except M2+− polymorphisms in the control population. This may be due to a low frequency of homozygote M2−−. Genotype and allele frequencies of APOA1 −75 G/A and M2+−− MspI polymorphisms in gallstone patients and controls are given in Tables 2 and 3, respectively.

Case-control analysis of genotype and allele

Genotype GG was found to be significantly higher (P=0.015; OR 1.559, 95% CI 1.087 to 2.236) in patients than in controls (64.90% versus 54.26%). Contrary to these results, the AA genotype was significantly lower (P=0.011) in patients than in controls (1.44% versus 5.99%) and was found to be protective (OR 0.230, 95% CI 0.067 to 0.786) against the disease (Table 2). After data were stratified based on sex, the GG genotype was found to pose a risk for the disease in males only (P=0.003; OR 2.656, 95% CI 1.373 to 5.138). In females, the GG genotype was not associated with the disease. The heterozygote GA genotype showed an association (P=0.031; OR 0.479, 95% CI 0.244 to 0.939) with the disease in the males, and the frequency of the GA genotype was significantly higher in controls than in patients (39.13% versus 23.53%). Although the frequency of the AA genotype was higher in controls than in patients, both in males and in females, the difference was not statistically significant.

The frequency of the G allele was significantly higher in gallstone patients (P=0.004; OR 1.561, 95% CI 1.150 to 2.119) than in controls (Table 3). Alternatively, the A allele was found to be protective against the disease (OR 0.641, 95% CI 0.451 to 0.87). Haplotypes were also constructed separately for the male and female populations (Table 5). In males, G-M2+−S1 was associated with a high risk (P=0.036; OR 1.593, 95% CI 1.029 to 2.464) for developing gallstone disease. A-M2+−S1 was also significantly (P=0.002; OR 0.370, 95% CI 0.197 to 0.695) associated with the disease. Haplotypes A-M2−−S1 and A-M2−−S2 were present only in controls, and haplotype A-M2−−S2 was absent in males and in female controls also. In females, G-M2−−S2 was absent in both patients and controls, and no other haplotype frequency showed a significant difference between patients and controls.

S1 and S2 alleles were defined based on the absence or presence, respectively, of the SstI restriction site. In patients, the frequency of the S1S1 genotype was lower (48.02% versus 55.38%) and the frequency of the S1S2 genotype was higher (43.07% versus 37.03%) than in controls, but the differences were not statistically significant. Genotype S2S2 showed an almost similar frequency in both groups. Sex-based stratification of the study population revealed that the APOC3 SstI polymorphism was not associated with gallstone disease in either sex (Table 4).

Frequency of the S1 allele was lower and frequency of the S2 allele was higher in patients than in controls (Table 4). Stratification of the study population into male and female categories showed the same trends of frequency distribution in patients and controls, but the difference was not statistically significant.

Case-control analysis of haplotype

APOA1−75−−APOA1+83−APOC3 haplotype: Haplotypes were constructed for all three polymorphisms present at the APOA1−75−−APOA1+83−APOC3 loci in the total population (Figure 1). Haplotype A-M2−−S1 was significantly (P=0.001) associated with gallstone disease. Haplotypes were also constructed separately for the male and female populations (Table 5). In males, G-M2−−S1 was associated with a high risk (P=0.036; OR 1.593, 95% CI 1.029 to 2.464) for developing gallstone disease. A-M2−−S1 was also significantly (P=0.002; OR 0.370, 95% CI 0.197 to 0.695) associated with the disease. Haplotypes A-M2−−S1 and A-M2−−S2 were present only in controls, and haplotype A-M2−−S2 was absent in males and in female controls also. In females, G-M2−−S2 was absent in both patients and controls, and no other haplotype frequency showed a significant difference between patients and controls.

APOA1−75−−APOA1+83−APOC3 SstI haplotype: Analysis of the APOA1−75−−APOA1+83−APOC3 SstI loci in the total population showed that A-S1 (P=0.002) was protective (OR 0.601, 95% CI 0.435

TABLE 1

Demographic profile of gallstone patients and controls

<table>
<thead>
<tr>
<th>Demographic profile</th>
<th>Patients</th>
<th>Controls</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Age, years (mean ± SD)</td>
<td>47.1±13.20</td>
<td>43.9±11.46</td>
<td>0.500</td>
</tr>
<tr>
<td>Sex, male:female</td>
<td>69:145</td>
<td>116:206</td>
<td>0.368</td>
</tr>
<tr>
<td>Body mass index, kg/m² (mean ± SD)</td>
<td>22.9±3.90</td>
<td>23.1±3.85</td>
<td>0.732</td>
</tr>
</tbody>
</table>
Table 2

APOA1 gene –75 and +83 MspI polymorphism genotype frequencies in gallstone patients and controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Male</th>
<th>Female</th>
<th>P (95% CI)</th>
<th>Male</th>
<th>Female</th>
<th>P (95% CI)</th>
<th>Male</th>
<th>Female</th>
<th>P (95% CI)</th>
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<td>Control</td>
<td>Patient</td>
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<td>Control</td>
<td>Patient</td>
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<td>Patient</td>
<td>Control</td>
</tr>
<tr>
<td>APOA1 –75 guanine (G) to adenine (A) polymorphism</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>GG</td>
<td>155 (75.00)</td>
<td>192 (60.00)</td>
<td>0.015</td>
<td>1.599</td>
<td>(1.087–2.336)</td>
<td>131 (75.00)</td>
<td>187 (54.00)</td>
<td>0.015</td>
<td>2.147</td>
<td>(1.475–3.106)</td>
<td>56 (60.00)</td>
<td>65 (54.00)</td>
</tr>
<tr>
<td>GA</td>
<td>18 (9.00)</td>
<td>20 (6.00)</td>
<td>0.158</td>
<td>0.595</td>
<td>(0.254–1.408)</td>
<td>4 (9.00)</td>
<td>7 (5.00)</td>
<td>0.158</td>
<td>1.250</td>
<td>(0.500–3.103)</td>
<td>16 (9.00)</td>
<td>23 (6.00)</td>
</tr>
<tr>
<td>AA</td>
<td>1 (0.50)</td>
<td>1 (0.30)</td>
<td>0.011</td>
<td>0.103</td>
<td>(0.025–0.440)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0.011</td>
<td>0.103</td>
<td>(0.025–0.440)</td>
<td>0 (0.00)</td>
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Table 3

APOA1 gene –75 and +83 MspI polymorphism allele frequencies in gallstone patients and controls

<table>
<thead>
<tr>
<th>Allele</th>
<th>Male</th>
<th>Female</th>
<th>P (95% CI)</th>
<th>Male</th>
<th>Female</th>
<th>P (95% CI)</th>
<th>Male</th>
<th>Female</th>
<th>P (95% CI)</th>
<th>Male</th>
<th>Female</th>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>340 (81.73)</td>
<td>470 (74.13)</td>
<td>0.004</td>
<td>1.561</td>
<td>(0.666–2.364)</td>
<td>118 (86.76)</td>
<td>167 (72.61)</td>
<td>0.002</td>
<td>2.473</td>
<td>(0.686–9.287)</td>
<td>222 (79.29)</td>
<td>303 (75.00)</td>
</tr>
<tr>
<td>A</td>
<td>76 (18.27)</td>
<td>164 (25.87)</td>
<td>0.004</td>
<td>0.641</td>
<td>(0.472–0.870)</td>
<td>18 (13.24)</td>
<td>27 (15.39)</td>
<td>0.002</td>
<td>0.485</td>
<td>(0.228–0.718)</td>
<td>58 (20.71)</td>
<td>101 (25.00)</td>
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</table>

Table 4

APOC3 SstI polymorphism genotype and allele frequencies in total subjects and stratified in male and female patients and controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Male</th>
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<th>Female</th>
<th>P (95% CI)</th>
<th>Male</th>
<th>Female</th>
<th>P (95% CI)</th>
<th>Male</th>
<th>Female</th>
<th>P (95% CI)</th>
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<td>Control</td>
<td>Patient</td>
<td>Control</td>
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<td>Control</td>
<td>Patient</td>
<td>Control</td>
<td>Patient</td>
<td>Control</td>
</tr>
<tr>
<td>S1S1</td>
<td>97 (48.02)</td>
<td>175 (55.38)</td>
<td>0.102</td>
<td>0.744</td>
<td>(0.522–1.061)</td>
<td>30 (46.15)</td>
<td>66 (58.41)</td>
<td>0.114</td>
<td>0.610</td>
<td>(0.330–1.219)</td>
<td>67 (48.91)</td>
<td>109 (53.69)</td>
</tr>
<tr>
<td>S1S2</td>
<td>87 (43.07)</td>
<td>117 (37.03)</td>
<td>0.170</td>
<td>1.287</td>
<td>(0.898–1.845)</td>
<td>29 (44.62)</td>
<td>37 (32.74)</td>
<td>0.114</td>
<td>1.655</td>
<td>(0.884–3.209)</td>
<td>58 (20.71)</td>
<td>101 (25.00)</td>
</tr>
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</table>

Table 5

APOC3 SstI polymorphism allele frequencies in total subjects and stratified in male and female patients and controls

<table>
<thead>
<tr>
<th>Allele</th>
<th>Male</th>
<th>Female</th>
<th>P (95% CI)</th>
<th>Male</th>
<th>Female</th>
<th>P (95% CI)</th>
<th>Male</th>
<th>Female</th>
<th>P (95% CI)</th>
<th>Male</th>
<th>Female</th>
<th>P (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>281 (89.55)</td>
<td>467 (73.89)</td>
<td>0.128</td>
<td>0.607</td>
<td>(0.612–1.064)</td>
<td>89 (88.46)</td>
<td>169 (74.78)</td>
<td>0.199</td>
<td>0.732</td>
<td>(0.453–1.179)</td>
<td>192 (70.07)</td>
<td>286 (73.40)</td>
</tr>
<tr>
<td>S2</td>
<td>123 (30.45)</td>
<td>185 (26.11)</td>
<td>0.128</td>
<td>1.239</td>
<td>(0.940–1.633)</td>
<td>41 (31.54)</td>
<td>57 (25.22)</td>
<td>0.199</td>
<td>1.399</td>
<td>(0.848–2.199)</td>
<td>82 (29.93)</td>
<td>108 (26.60)</td>
</tr>
</tbody>
</table>

*Total number of chromosomes: –75 75 base pairs upstream; APOA1 Apolipoprotein A1; APOC3 Apolipoprotein C-3; S1 Allele with no SstI restriction site; S2 Allele with an SstI restriction site
Gallstone disease is a major problem in India and, with privileged circumstances and urbanization in rural villages, its rate may increase in the future. Alterations in lipids have been associated with disease but the mechanism is unknown. Genetic association studies in humans have the statistical power to reveal the contribution of risk alleles to a polygenic disease like gallstone formation. The low HDL cholesterol and high triglyceride concentrations reported in gallstone patients led us to search for a possible association between genetic polymorphisms and gallstone disease in the Indian population. The most important finding of this study was that the –75 G/A polymorphism alone could explain 1.1% of cases, suggesting a significant contribution of this polymorphism to genetic risk. This polymorphism was definitely a risk factor for gallstone disease because an adjusted OR of the G allele for the environmental factors of age, sex, and BMI and controls also did not reveal any difference (data not shown).

### Risk assessment

To determine the contribution of different alleles to the risk of gallstone disease, logistic regression analysis was used (Table 6). Different models were constructed to determine the contribution of risk alleles alone and in association with environmental factors for all three polymorphisms. The APOAI G allele gave 1.4% variance when adjusted for age and sex, and with inclusion of BMI in the model, it predicted 1.6% variance for gallstone disease. In the APOAI M2+/– polymorphism, the M2+ allele, and in the APOC3 SstI polymorphism, the S2 allele did not show any significant risk, even after adjustment for the covariates of age, sex, and BMI.

### DISCUSSION

Gallstone disease is a major problem in India and, with privileged circumstances and urbanization in rural villages, its rate may increase in the future. Alterations in lipids have been associated with disease but the mechanism is unknown. Genetic association studies in humans have the statistical power to reveal the contribution of risk alleles to a polygenic disease like gallstone formation. The low HDL cholesterol and high triglyceride concentrations reported in gallstone patients led us to search for a possible association between genetic polymorphisms and gallstone disease in the Indian population. The most important finding of this study was that the –75 G/A polymorphism alone could explain 1.1% of cases, suggesting a significant contribution of this polymorphism to genetic risk. This polymorphism was definitely a risk factor for gallstone disease because an adjusted OR of the G allele for the environmental factors of age, sex, and BMI and controls also did not reveal any difference (data not shown).

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TABLE 6
Adjusted ORs for gallstone disease according to alleles

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Model</th>
<th>P</th>
<th>OR</th>
<th>95% CI</th>
<th>R² × 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOA1 –75 G/A</td>
<td>G allele + age + sex</td>
<td>0.004</td>
<td>1.562</td>
<td>1.150–2.122</td>
<td>1.4</td>
</tr>
<tr>
<td>APOA1 M2*/+</td>
<td>M2* allele + age + sex + BMI</td>
<td>0.371</td>
<td>1.314</td>
<td>0.723–2.386</td>
<td>0.5</td>
</tr>
<tr>
<td>APOA1 M2+ allele + 0.371 1.314 0.723–2.386 0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APOC3 SstI</td>
<td>S2 allele +age + sex</td>
<td>0.123</td>
<td>1.243</td>
<td>0.943–1.640</td>
<td>0.7</td>
</tr>
<tr>
<td>APOC3 SstI</td>
<td>S2 allele + age + sex + BMI</td>
<td>0.116</td>
<td>1.249</td>
<td>0.947–1.649</td>
<td>0.7</td>
</tr>
</tbody>
</table>

−75 75 base pairs upstream; APOA1 Apolipoprotein A1; APOC3 Apolipoprotein C-3; BMI Body mass index; G/A Guanine (G) to adenine (A) polymorphism; R² ×100 Part of variance explained by predictors

(30). This G to A substitution is between the CACAT sequence and the TAAATA box of the transcription start site of APOA1 and creates a 6 bp perfect repeat (CAGGCC) which has homology to known nuclear-protein binding sites (31,32). On the other hand, studies surrounding transcription rates have associated the APOA1 A allele with a four- to sevenfold increase in the transcription rate of APOA1 in vitro (28,33). Therefore, we hypothesize that in APOA1 A allele carriers, the level of APOA1 protein is increased, which acts as an antinucleating agent and provides protection against gallstone formation.

Sex-specific differences were clearly seen in the distribution of the –75 G/A polymorphism between gallstone patients and controls, and the association was observed only in males. A meta-analysis has also shown a more apparent effect of this polymorphism on lipids in male subjects (14). This sex-specific difference can be attributed to the interaction between sex-specific hormones and genetic variants. To date, there is no study showing the molecular mechanism of gene-hormone interactions in gallstone disease.

The present study also considered the APOA1 M2*/+ polymorphism in gallstone patients for the first time. Though this polymorphism was not associated with gallstone disease in the present study, trends toward an association were observed. A larger sample size may reveal a significant association with gallstone disease.

Another widely studied polymorphism of the APOA1-C3 cluster is the SstI polymorphism in the 3' untranslated region of APOC3. The S2 allele has also been associated with elevated triacylglycerol, cholesterol and APOC3 concentration, and increased coronary artery disease risk (34-36). In our study, the SstI polymorphism in APOC3 was not found to be associated with gallstone disease. Regression analysis for adjustment of environmental factors only revealed trends toward an association (Table 6). A recent study using twins also reported a significant contribution of environmental factors to the development of gallstone disease (5).

In gallstone disease, multiple candidate genes are proposed (37) and each gene may have multiple genetic variants. Some alleles may provide protection and others may be associated with a higher risk for gallstone disease. To estimate the overall risk for gallstone disease in an individual, it is pertinent to analyze all genetic polymorphisms simultaneously. Therefore, the haplotype analysis is more accurate than the genotype or allele analyses. It was found that haplotype frequencies of APOA1-C3 in males were significantly different between patients and controls (Table 5). The combined effect of all three polymorphisms of APOA1-C3 suggests that the major contribution is from the APOA1 –75 G/A polymorphism. However, a significant association of APOA1 haplotype (G-M2*) with gallstone disease was observed, where measures of association (P=0.0001, OR 3.173) were remarkably higher (Table 5) than the APOA1 –75 G/A polymorphism alone. Although APOA1 M2*/+ was not significantly associated with disease it did show a weak association (P=0.088). This indicates that the APOA1 M2*/+ polymorphism also contributes toward risk, and studies in large populations might reveal this.

By virtue of the APOA1 –75 G/A polymorphism, which was significantly associated with gallstone disease, the maximum variance observed in the present study was 1.6%, which indicates that other genetic factors also contribute to the risk of developing gallstone disease. A recent study involving twins detected a 25% variance for heritability (5). The genes involved in gallbladder motility (cholecystokinin A receptor and cholecystokinin), cholesterol synthesis (3-hydroxy-3-methylglutaryl-coenzyme A reductase), bile acid synthesis (cholesterol 7-α hydroxylase) and mucins are important candidates for genetic studies in gallstone disease. Identification of all risk alleles will provide a definite contribution of risk due to genetic factors. Because this is the first study of its kind, additional studies in different populations with a large sample size are required for confirmation of the present study’s results.

CONCLUSIONS

The APOA1 –75 G/A polymorphism is associated with gallstone disease and shows sex-specific differences. APOA1 M2*/+ and APOC3 SstI polymorphisms may not be associated with gallstone disease. Haplotype analysis is a better predictor of risk for gallstone disease than the genotype or allele analyses.

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