The overexpression of Sterol Carrier Protein 2 gene in patients of hereditary cholesterol gallstones

YunFeng Cui¹, ZhongLian Li¹, YanFeng Jia¹, DongHua Li¹, Ju Zhang², NaiQiang Cui¹*  
¹Department of Surgery, Tianjin Nankai Hospital, Nankai Clinical School of Medicine, Tianjin Medical University, 122 Sanwei Road Nankai District, Tianjin 300100, China  
²Institute of Molecular Biology, Nankai University, 94 Weijin Road Nankai District, Tianjin 300071, China  
*Corresponding author  
Email addresses:  
YFC: yunfengcuidoctor@yahoo.com.cn  
ZLL: lizhonglian@medmail.com.cn  
YFJ: jyf102030@qq.com  
DHL: NKLDH@163.com  
JZ: zhangju@nankai.edu.cn  
NQC: yfcuinlk@hotmail.com  

Abstract  

Background  
Lithogenic bile is the major cause of cholesterol gallstones, but its pathogenesis is not well understood, the hypersecretion of biliary cholesterol is believed to be one important cause of lithogenic bile. SCP2 participates in cholesterol trafficking and metabolism of hepatocytes and may play a key role in cholesterol gallstone formation.  

Methods  
To investigate the expression level of liver SCP2 gene in hereditary and non-hereditary cholesterol gallstone patients and non-cholesterol gallstone patients. At the same time, to elucidate the lithogenic changes of gallbladder bile from gallstone patients. The expression level of SCP2 mRNA was studied in 28 hereditary, and 30 non-hereditary, cholesterol gallstone patients, and 32 non-cholesterol gallstone patients by Reverse Transcription Polymerase Chain Reaction (RT-PCR). At the same time the level of liver SCP2 protein was detected in above patients by Western blotting method. The bile was also analyzed by means of biochemical techniques and the Cholesterol Saturation Index (CSI) was calculated at the same time.  

Results  
The expression of SCP2 mRNA and protein was increased significantly in cholesterol gallstone patients as compared with non-cholesterol gallstone patients. Moreover, there is also a significant increase in hereditary cholesterol gallstone patients, as
compared with non-hereditary cholesterol gallstone patients. We found significant differences in CSI between cholesterol gallstone patients and non-cholesterol gallstone patients, but there was no significant difference in CSI between hereditary and non-hereditary cholesterol gallstone patients.

**Conclusions**

SCP2 was overexpressed in cholesterol gallstone patients, which indicated that SCP2 might be one important cause of cholesterol gallstones. The over expression of SCP2 might be a hereditary factor during the formation of cholesterol gallstones, and the formation of stones was always accompanied by the increase of bile lithogenicity.

**Background**

Hypersecretion of cholesterol in bile leading to the formation of lithogenic bile is believed to be the major cause of cholesterol gallstones [1,2]. Sterol carrier protein 2 (SCP2), also called nonspecific lipid transfer protein, is a 13.2 KD base protein and exists in peroxisome, mitochondria, endoplasmic reticulum and cytoplasm [3,4,5]. As a moderating factor of cholesterol metabolism, it is involved in the biosynthesis of cholesterol [6,7,8] and the trafficking of cholesterol to bile acid [9,10], cholesteryl ester [11] and sterols [12]. As a transporting tool, on the other hand, the protein participates in the transportation of cholesterol inside the cell and through the cytoplasm membrane [13,14] as well as the rapid transportation of the newly synthesized cholesterol from endoplasmic reticulum into the bile without the intervention of cytomicrotubule system and Golgi bodies [15]. Hence, hypersecretion of biliary cholesterol with the formation of lithogenic bile may explain the mechanism of the formation of cholesterol stones in the gallbladder.

We accumulated 21 cholesterol gallstone genealogies and undertook a study to investigate the expression of SCP2 gene in the liver tissue of hereditary and non-hereditary cholesterol gallstone patients and non-cholesterol gallstone patients, using reverse transcription-polymerase chain reaction and western blotting. The bile was also analyzed by means of biochemistical techniques and Cholesterol Saturation Index (CSI) was calculated at the same time.

**Methods**

**Cases Selection**

**Hereditary gallstone group:** Genealogies accorded with the criterion that there are two generations with cholesterol gallstones in three consanguinity generations. Patients with cholesterol stones in the gallbladder, the cholesterol contents >50%.

**Non-hereditary cholesterol gallstone group:** Patients with cholesterol stones in the gallbladder, the cholesterol contents>50%.
Control group: Patients with primary intrahepatic cholangiolithiasis, the cholesterol contents < 20% in the stone and/or patients with peptic ulcer, cancer of the stomach or of the colon in whom no gallstones are verified by ultrasound diagnosis.

Exclusion criteria: Diabetics and patients with other endocrine metabolism disorders; obese individuals; and patients with other diseases of the liver and the gallbladder.

All patients were enrolled in Tianjin Nankai Hospital from August 2003 to August 2008 and they were all inpatients and had operations. The first group consists of 28 patients with cholesterol stones in the gallbladder, 15 females and 13 males, aging between 25-80 years old. The stones appeared yellow or light yellow in color, with smooth or nodular surfaces. The second group consists of 30 patients with cholesterol stones in the gallbladder, 16 females and 14 males, aging between 20-80 years old. The control group consists of 32 patients, including 16 females and 16 males, aging between 15-78 years old. 8 patients in control group were primary hepatobiliary-lithiasis, with the stones brown or dark-brown in color, friable, consistent with bile pigment stones, 5 with gastric cancer,11 with non-cholesterol polyp in the gallbladder, and 8 with pancreatic cancer. There were no significant differences between the three groups with the age and the body mass index (P>0.05).

Bile and Liver Sampling
Patients fasted for 12 hours before the operation, general anesthesia was administered, a liver specimen of 50 mg was obtained, and bile was collected from gallbladder. Bile was kept at -20°C, whereas liver tissues were stored at -70°C until processed. The biopsy was permitted by Hospital Ethics Committee and consensuses were obtained from the patient and family members for the biopsy.

Identification of Cholesterol Stones
Determination of cholesterol content in gallstones was done as follows: stones obtained in the operation were rinsed with water and put in the dryer, until a constant weight was obtained. Then the stones were ground, dried naturally for 12 hours. A 10 mg sample of gallstone powder was weighed and dissolved in 5 ml anhydrous alcohol, stirred for 3 minutes, and centrifuged at 3000 rpm for 5 minutes. The supernatant was collected for further analysis, another 10 mg sample was accurately weighed and dissolved in 5ml chloroform, stirred for 3 minutes and centrifuged at 3000 rpm for 5 minutes, and the supernatant was collected for analysis. The cholesterol and calcium bilirubinate were measured by enzyme method. The ratio of cholesterol content to calcium bilirubinate content was calculated and specimens with a ratio of >0.5 were classified as cholesterol stones, and those with a ratio of <0.5 as pigment stones.

Reverse-Transcription Polymerase Chain Reaction
Total RNA was prepared from liver tissue using Trizol method and 1000ng RNA was reverse-transcribed using random primer hexamers. Reverse-transcription polymerase
chain reaction (RT-PCR) was performed using primers based on human complementary DNA sequences available through Gene Bank databases. Target gene and $\beta_2$-Microglobulin gene as an internal control was amplified at the same time with the same conditions. RT-PCR conditions, primers, and PCR fragment sizes are shown in Table 1. After electrophoresis PCR products were quantified by the Gene Genius Gel Documentation and Analysis System, measurements were normalized by the corresponding $\beta_2$-Microglobulin signal.

**Western Blot**

The tissues were lasered with the lysis buffer (50 mM Tris PH7.4, 150 mM NaCl, 0.5% Triton X-100, 1 mM EDTA, 1 mM PMSF). The total protein was separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis and electrophoretically transferred onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA). After blocking non-specific binding sites with 5% non-fat milk, the membrane was incubated with primary antibodies for 1 h at room temperature. The primary antibodies used in this experiment were as follows: anti-SCP2 antibody (Donated by Professor Schroeder F., Texas A&M University, 1:1000 dilution) and anti-GAPDH antibody (Santa Cruz Biotechnology, 1:2000 dilution). After washing, the blots were incubated with horseradish peroxidase-conjugated antimouse or anti-rabbit IgG, and immunoreactive bands were visualized with Western Blotting Luminol Reagent kit (Santa Cruz Biotechnology Inc).

**Bile Biochemical Analyses**

Biliary Cholesterol, Phospholipids, and Total Bile Acids concentrations were measured using enzymatic kits (Zhongsheng Bioengineering High-Tech Co.).

**Data Analysis**

All data are expressed as mean ± standard deviation. Differences, between control and experimental groups, were evaluated by one-way analysis of variance (ANOVA). $P<0.05$ was considered statistically significant ($a = 0.05$).

**Results**

**The expression of SCP2 between hereditary gallstone group, non-hereditary gallstone group and control group**

To explore the potential mechanisms of SCP2 gene, we evaluated mRNA and protein expression levels of it, which was the main cholesterol transporter and modulator in hepatocytes. As shown in Fig. 1, we found significant differences about SCP2 mRNA levels in hereditary and non-hereditary cholesterol gallstone patients compared with control group (0.8908±0.1649, 0.7503±0.1004 and 0.5205±0.1900, $p<0.05$). Moreover, there is also a significant difference in SCP2 mRNA level between hereditary and non-hereditary cholesterol gallstone patients. From the results of western blotting, we also found the same changes of protein level in these three groups (1.5898±0.0316, 1.3032±0.0664 and 0.7637±0.0258, $p<0.05$ ).
Changes of bile components and lithogenicity between hereditary, non-hereditary cholesterol gallstone patients and control groups

We assayed the bile samples and got the concentrations of cholesterol, phospholipids and bile acids in bile from gallbladder and calculated the CSI using the Carey table. As shown in Table 2, we found, Cholesterol level of hereditary, and non-hereditary cholesterol gallstone patients, were both higher than the control group. Total bile acids levels of hereditary, and non-hereditary cholesterol gallstone patients, were both lower than control group, there was no significant difference in Phospholipids between three groups. There were significant differences in CSI between cholesterol gallstone patients, and non-cholesterol gallstone patients, but there was no significant difference in CSI between hereditary, and non-hereditary cholesterol gallstone patients.

Discussion

Cholesterol gallstone formation is a complicated process, involving a variety of factors. The abnormal metabolism of liver cholesterol and supersaturation of bile cholesterol are the first and most important conditions in stone formation. In recent years some scholars have taken a series of epidemiological studies about cholelithiasis [16,17,18] and findings show: gallstone disease is a multi-gene genetic diseases with a hereditary tendency and genetic characteristics of autosomal dominant delay. The comprehensive effects of related genes and environmental factors play an important role in the pathogenesis of gallstone formation.

Animal experiments confirmed that SCP2 was an essential factor in the transportation of newly synthesized cholesterol into bile, and it could rapidly transfer cholesterol from endoplasmic reticulum directly into the bile without the involvement of the cellular microtubule system and the Golgi [19]. Fuchs, et al [20,21] observed the phenomenon in the stone susceptible mice that SCP2 protein and SCP2 mRNA rose at the same time and thought that the transcriptional upregulation of SCP2 led to the higher level of SCP2 in liver cell, then resulting in the increment of liver cholesterol and bile cholesterol and promoting the gallstone formation. Further studies on susceptible mice found that Lith 1 gene might resulted in overexpression of SCP2 mRNA and SCP2 during the formation of gallstones.

In our former experiments, Zhang, et al [22] found that there was difference in the expression of SCP2 mRNA between cholesterol gallstone patients and the control group (P<0.05), which was similar to what was observed in susceptible mice by Fuchs, and Wang, et al also had the same results as our work. Cui, et al [23] increased the research cases and further confirmed that the expression of SCP2 mRNA was increased more significantly in patients with cholesterol gallstone than in patients with non-cholesterol gallstone. We also found there was a phenomenon that some gallstone patients gathered in some families and the people with this family
background were more susceptible to form stones than those without the family background. So in this study we enrolled the patients with family history as one group and enlarged the sample size on the basis of former work.

In this research, we found that the expression of SCP2 mRNA was higher in non-hereditary cholesterol gallstone group than that in the control group. In order to verify the reliability of the change of the SCP2 transcription level, we used the western blotting to detect the change of SCP2 protein level in the same individuals and found that the change of protein level was consistent with that of mRNA level, which was similar to our former results[22,23]. We also found that SCP2 mRNA and protein levels of hereditary cholesterol gallstone group were higher than those of non-hereditary cholesterol gallstone group and the difference was significant (P <0.05); therefore, we thought that SCP2 might be one of genetic factors in gallstone disease. SCP2 might increase the cholesterol concentration and the bile lithogenicity through the following processes: promote the formation of cholesterol from 7-dehydro-cholesterol, inhibit 7alpha-hydroxylase activity and in turn decrease the formation of bile acids from cholesterol, inhibit ACAT activity, decrease the formation of cholesterylester from cholesterol, inhibit HDL-cholesterol secretion and HDL receptor expression, and increase HDL-cholesterol concentration. Therefore, we believed that SCP2 could increase the bile cholesterol, reduce the bile acid in the bile and expedite the formation of lithogenic bile and the precipitation of cholesterol crystals, which provided the necessary conditions for the formation of stones.

We also found that the bile lithogenicity of cholesterol gallstone patients was higher than that of control group, but there was no significance difference in the bile lithogenicity between hereditary and non-hereditary cholesterol gallstone groups, so we could draw a conclusion that the formation of stones was always accompanied by the increase of bile lithogenicity. So if we combined the results of the changes of SCP2 expression with the results of the analysis of bile lithogenicity, we could find that SCP2 might influence the susceptibility to gallstones of different people under the same life environment.

**Conclusions**

The followings could be concluded from this study: over expression of SCP2 might be one of the important causes of cholesterol stones in the gallbladder, SCP2 might be one of the major pathogenic genes of cholesterol cholecystolithiasis, and the formation of stones is always accompanied by the increase of bile lithogenicity.

**Competing interests**

The authors declare that they have no competing interests.
Authors' contributions

YFC performed the majority of experiments; DHL and YFJ took part in some experiments; NQC and JZ involved in critical reading and helpful discussion of the manuscript; ZLL provided the collections of human liver and bile samples; YFC and NQC designed the study and wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgements

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References

Three groups were compared and $F=11.089$, $P<0.05$; SCP2 mRNA expression level of hereditary, and non-hereditary cholesterol gallstone patients, were $0.8908 \pm 0.1649$, $0.7503 \pm 0.1004$ separately, which were both higher than control group $0.5205 \pm 0.1900$, $P<0.05$. SCP2 mRNA expression level of hereditary cholesterol gallstone patients, was higher than control group, and the difference was statistically significant, $P<0.05$. 

Figure 1 The expression of SCP2 mRNA between hereditary gallstone group, non-hereditary gallstone group and control group

![Figure 1](image-url)
Figure 2 The expression of SCP2 protein between hereditary gallstone group, non-hereditary gallstone group and control group (control group, non-hereditary group, hereditary group)

Three groups were compared and F=1084.98, P<0.05; SCP2 protein level of hereditary, and non-hereditary cholesterol gallstone patients were 1.5898±0.0316, 1.3032±0.0664 separately, which were both higher than control group 0.7637±0.0258, P<0.05. SCP2 protein level of hereditary cholesterol gallstone patients was higher than control group, and the difference was statistically significant, P<0.05.
Cholesterol of three groups were compared and F=84.230, P <0.05. Cholesterol level of hereditary, and non-hereditary cholesterol gallstone patients, were both higher than control group, P <0.05. But there was no significant difference in cholesterol between hereditary, and non-hereditary cholesterol gallstone patients. Total Bile Acids of three groups were compared and F=17.382, P <0.05 total bile acids level of hereditary, and non-hereditary cholesterol gallstone patients were both lower than control group, P <0.05 but there was no significant difference in Total bile acids between hereditary and non-hereditary cholesterol gallstone patients. Phospholipids of three groups were compared and F=0.492, P>0.05 and there was no significant difference in Phospholipids between three groups.
Figure 4 Comparison of gallbladder bile CSI between hereditary, non-hereditary cholesterol gallstone patients and control group

CSI of three groups were compared and F=49.166, P<0.05. CSI of hereditary, and non-hereditary cholesterol gallstone patients, were both higher than control group, P <0.05 but, there was no significant difference in CSI between hereditary, and non-hereditary cholesterol gallstone patients.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers</th>
<th>Fragment Size (base pairs)</th>
<th>Amplification Times and Temperatures</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCP2</td>
<td>Upper: ATGGGGTTTCCGGAAGCCGCTGCCAGTT</td>
<td>432</td>
<td>1 min at 94°C</td>
</tr>
<tr>
<td></td>
<td>Lower: TCAGAGCTTAGCGTTGCCTGGCTG</td>
<td></td>
<td>1 min at 58°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 min at 72°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>22 cycles</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 min at 94°C</td>
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<tr>
<td>β2-Microglobulin</td>
<td>Upper: ATGCCTGCGTGTGGAACCATGT</td>
<td>285</td>
<td>1 min at 58°C</td>
</tr>
<tr>
<td></td>
<td>Lower: AGAGCTACCTGTGGAGCAACCT</td>
<td></td>
<td>1 min at 72°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>21 cycles</td>
</tr>
</tbody>
</table>
Table 2 The concentrations of Cholesterol, Total Bile Acids and Phospholipids in gallbladder bile

<table>
<thead>
<tr>
<th>group</th>
<th>Cholesterol (mmol/L)</th>
<th>Total Bile Acids (mmol/L)</th>
<th>Phospholipids (mmol/L)</th>
<th>CSI d</th>
</tr>
</thead>
<tbody>
<tr>
<td>control group</td>
<td>3.0620±0.7053</td>
<td>14.6700±1.3316</td>
<td>8.4880±0.8190</td>
<td>0.7342±0.1550</td>
</tr>
<tr>
<td>non-hereditary</td>
<td>3.8333±0.7217**</td>
<td>11.1667±1.2010**</td>
<td>8.8933±1.1162**</td>
<td>1.5190±0.2715**</td>
</tr>
<tr>
<td>hereditary group</td>
<td>3.9200±0.7229*</td>
<td>11.0900±1.2038*</td>
<td>8.8600±0.8050*</td>
<td>1.5810±0.2400*</td>
</tr>
</tbody>
</table>

a*compared with control group $P<0.05$, compared with non-hereditary group $P>0.05$; **compared with control group $P<0.05$

b*compared with control group $P<0.05$, compared with non-hereditary group $P>0.05$; **compared with control group $P<0.05$

c*compared with control group $P>0.05$, compared with non-hereditary group $P>0.05$; **compared with control group $P>0.05$

d*compared with control group $P<0.05$, compared with non-hereditary group $P>0.05$; **compared with control group $P<0.05$