Original article

The association between TCM syndromes and SCAP polymorphisms in subjects with non-alcoholic fatty liver disease

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Abstract

Introduction: In Western medicine, non-alcoholic fatty liver disease (NAFLD) is diagnosed by imaging, histology and biochemical parameters. Traditional Chinese Medicine (TCM) uses unique diagnostic techniques to classify NAFLD into subtypes based on different TCM symptoms (syndrome classification). Sterol regulatory element-binding protein (SREBP), also known as SREBP cleavage-activating protein or SCAP is encoded by the SCAP gene SCAP genes have important functions in defining genetic susceptibility to NAFLD. This study investigated whether the polymorphisms of SREBF-1, SREBF-2, and SCAP genes were associated with the TCM syndromes of NAFLD.

Materials and methods: Fourteen tag single nucleotide polymorphisms (SNPs) of SREBF-1, SREBF-2, and SCAP were chosen for our study We genotyped and analyzed 100 healthy control subjects and 211 NAFLD subjects who were classified by TCM into two groups, namely, deficiency syndrome group and excess syndrome group.

Results: The results showed that rs12636851 SNP of SCAP exhibited a significant genotype and allelic variation between the deficiency syndrome and healthy control subjects, as well as between the deficiency and excess syndrome subjects In the deficiency syndrome group, the subjects who had the CC or TC genotype of SCAP rs12636851 had a threefold elevated risk for NAFLD compared with the TT genotype (adjusted OR, 3107; 95% CI, 1023–9433, P = 0.045; adjusted OR, 2970; 95% CI, 1121–7864, P = 0.028).

Discussion: We speculate that the SCAP rs12636851 SNP in the deficiency syndrome subjects affects the cholesterol-sensing function of SCAP, increasing cholesterol and fatty acid synthesis in liver Therefore, this SNP may help in the understanding of the genetic basis of NAFLD patients with deficiency syndrome and in the development of personalized medical care.

Keywords: Non-alcoholic fatty liver disease; Genetic polymorphism; SCAP; Traditional Chinese Medicine; TCM syndrome classification

Introduction

Non-alcoholic fatty liver disease (NAFLD) is recognized as one of the most common causes of chronic liver disease worldwide [1]. The prevalence of NAFLD is about 15–30% in the general population of various countries [2]; approximately 20% in China [3,4]. NAFLD is diagnosed by imaging or histology as well as biochemical parameters in western medicine. However, in clinical practice patients with NAFLD present with different clinical symptoms. Traditional Chinese Medicine (TCM) uses a unique diagnostic technique to classify NAFLD into subtypes based on these different TCM symptoms (called as
TCM syndrome classification). This method of classifications limits the clinical heterogeneity of NAFLD and provides a basis for developing a classified treatment protocol.

The pathogenesis of NAFLD is complex and multifactorial, as environmental and genetic factors interact with each other [5,6]. Environmental factors such as excessive calorie intake and a lack of daily physical activity are undoubtedly fuelling the epidemic of NAFLD [6,7]. However, environmental factors are not solely responsible for the NAFLD problem. In clinical practice, there are individual variations in susceptibility to the development of NAFLD that is, some individuals develop NAFLD, whereas others remain unaffected even when sharing a similar moderate lifestyle. These observations suggest that innate, non-environmental factors make some individuals more susceptible to NAFLD. In the recent years, several genes have been suggested potentially associated with NAFLD-related traits in the general population [8–10]; however, the contribution of genetic polymorphisms to the disease susceptibility is still inconclusive. As cholesterol and fatty acid metabolism plays an important role in NAFLD pathogenesis [11], genetic variations in candidate genes related to dyslipidemia susceptibility may be associated with NAFLD. Sterol regulatory element-binding protein (SREBP) is one of the major regulators of lipid metabolism, especially in cholesterol and fatty acid synthesis [12]. SREBPs are produced from separated genes named sterol regulatory element-binding factors-1 (SREBF-1) and SREBF-2 [13]. The SREBP cleavage activating protein (SCAP) is involved in maturation of both SREBPs [14] and transports SREBPs from the endoplasmic reticulum to the Golgi complex. The SREBPs are subsequently activated and translocated into the nucleus. The SREBPs bind to SREBP response element to stimulate the expression of target genes, encoding enzymes for synthesis and uptake of cholesterol and triglyceride [15,16].

Therefore, SREBF-1, SREBF-2, and SCAP genes have important functions in defining genetic susceptibility to NAFLD. Moreover, the different genotypes of these genes can possibly distinguish the NAFLD subtypes. In the current study, we investigated whether the polymorphism of SREBF-1, SREBF-2, and SCAP genes are associated with the TCM syndromes of NAFLD.

Materials and methods

Subjects

This study was conducted at Longhua Hospital, which is affiliated to the Shanghai University of TCM and Fenglin Community Hospital in the Xuhui District of Shanghai, from August 2009 to May 2010. A total of 311 unrelated individuals were enrolled in this study, of the total number subjects, 211 individuals were diagnosed with NAFLD, and the remaining 100 individuals were selected as healthy control subjects with no history of fatty liver.

Clinical and laboratory evaluation

See Supplementary materials and methods.

Diagnostic criteria

NAFLD was diagnosed according to the guidelines for the diagnosis and treatment of NAFLDs issued by the Fatty Liver and Alcoholic Liver Disease Study Group of the Chinese Liver Disease Association (2008 and 2010) [17] (see Supplementary materials and methods).

In order to determine TCM syndromes, NAFLD subjects completed questionnaires which included 30 symptoms (Table 1), which were clustered into two specific groups using a K-means cluster analysis. Coincidentally, the two groups were similar to the deficiency syndrome and excess syndrome of the TCM theory based on “Textbooks for general tertiary education of Chinese medicine: diagnosis of Chinese medicine”, which was edited and authorized by Ministry of Health of China [18]. Symptoms less correlated to the TCM syndrome classification of NAFLD or did not correspond to the TCM theory were excluded (Supplementary Fig. S1). Finally, a 12-item scoring table for the TCM diagnosis of NAFLD was finalized; each diagnostic category comprised eight symptoms including 2 main symptoms and 6 minor symptoms (Supplementary Table S2). The two main symptoms each corresponded to two points and the other symptoms corresponded to one point. As a result, the category that received a score of five points or higher was considered to deficiency or excess syndrome. If two categories received a score of five points or higher, the patient was considered to have a deficiency and excess coexisting syndrome. If two categories received a score less than five points, the patient was considered to have no syndrome. The small population of the deficiency and excess coexisting syndrome and no syndrome (6.2% and 5.2%) in present study decreased the power to detect differences, thus were excluded in the analysis. Therefore, a total of 287 subjects with the deficiency or excess syndrome were enrolled in the following analysis (Fig. 1).

Tag SNPs selection

A tag SNP is a representative SNP in a region of the genome with high linkage disequilibrium, which could predict the rest of the SNPs with a small error. Thus, when performing a disease association study, the geneticist would experimentally test for association by considering only the tag SNPs, thereby considerably saving resources. We selected tag SNPs (tSNPs) using genotype data obtained from the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov) (release # 27/PhaseII+III Feb 09). This study aims to define a set of tSNPs that have an estimated $r^2 > 0.8$ compared with the untyped SNPs [19]. Using the Haploview 4.2 program (http://www.broad.mit.edu/haploview/haploview-downloads), we selected the tSNPs having a minor allele frequency of $>0.05$ in Chinese Han Beijing (CHB). Therefore, a total of 14 SNPs were chosen for this study.

SNP genotyping assays

SNPs were typed using iPLEX chemistry on a matrix-assisted laser desorption/ionization time-of-flight mass spectrometer
Table 1
Clinical and biological characteristics of subjects in four groups.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total NAFLD (n=187)</th>
<th>Healthy control (n=100)</th>
<th>NAFLD Xu pattern (n=91)</th>
<th>NAFLD Shi pattern (n=96)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female (%)</td>
<td>Age (years)</td>
<td>BMI (kg/m²)</td>
<td>FPG (mmol/L)</td>
</tr>
<tr>
<td></td>
<td>62.6</td>
<td>69.96 ± 8.70</td>
<td>26.07 ± 2.89</td>
<td>7.51 ± 2.29</td>
</tr>
<tr>
<td></td>
<td>57.0</td>
<td>66.65 ± 5.30*</td>
<td>22.82 ± 1.54**</td>
<td>5.13 ± 0.81**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13.9</td>
<td>7.13 ± 0.81**</td>
<td>5.13 ± 0.81**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.0</td>
<td>7.13 ± 0.81**</td>
<td>5.13 ± 0.81**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>63.7</td>
<td>70.89 ± 8.95</td>
<td>25.92 ± 2.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>61.5</td>
<td>69.08 ± 8.42</td>
<td>26.21 ± 2.82</td>
</tr>
</tbody>
</table>

BMI, body mass index; FPG, fasting plasma glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; VLDL-c, very-low-density lipoprotein cholesterol; ALT, alanine transaminase; AST, aspartate transaminase.

* P<0.05 vs. the healthy control group.

** P<0.01 vs. the healthy control group.

*** P<0.05 vs. the Shi pattern group.

Statistical analysis

The Hardy–Weinberg equilibrium was calculated using goodness-of-fit χ²-tests to compare the observed genotype frequencies with the expected genotype frequencies in the healthy control group. Continuous variables were expressed as mean ± standard deviation and the differences between groups were compared by Student’s t-test or Kruskal–Wallis test. Categorical variables were expressed as the number of cases and percentage. The results were then compared using the χ²-test or Fisher’s test. Odds ratios (ORs) and 95% confidence interval (CI) were calculated separately using binary logistic regression analysis adjusted for gender, age, smoking status, and BMI. We performed binary logistic regression analysis by assuming an additive and dominant model. An additive model referred to a variable coded 0, 1, 2 for the three genotypes AA (major homozygous), Aa (heterozygous), and aa (minor homozygous). The codes were then entered into binary logistic regression analysis as integers in the model. A dominant model coded the genotypes as 0 = AA (major homozygous) and 1 = Aa + aa (heterozygous combined with minor homozygous). Two-sided P values of <0.05 were considered statistically significant. All of these statistical analyses were conducted in SPSS version 17.0 (SPSS Software, Chicago, IL, USA).

Results

The clinical and laboratory characteristics of the four groups were listed in Table 1. Compared with the healthy controls, the NAFLD subjects including the excess and deficiency syndrome
groups exhibited higher values for body mass index (BMI), fasting plasma glucose (FPG), serum lipids, and blood pressure. In the NAFLD group, no significant differences were found between the excess and deficiency syndrome subjects for the clinical and laboratory characteristics, except for the slightly higher low-density lipoprotein cholesterol (LDL-c) in the deficiency syndrome group ($P = 0.039$).

All the SNPs passed the Hardy-Weinberg equilibrium test ($P > 0.05$, Table 2) in the healthy control group. The associations between the phenotypes and 14 SNPs were shown in Table 2 and Supplementary Tables 3 and 4. Only the SCAP rs12636851 SNP was found to be significantly associated with NAFLD in the deficiency syndrome group and the healthy control group in the additive model ($P = 0.041$, Table 2), the dominant model ($P = 0.018$, Table 2) and the genotypic distributions ($P = 0.004$, Table 2).

Our findings indicated that SCAP rs12636851 SNP exhibited a positive relationship with NAFLD in the deficiency syndrome subjects. Regardless of the syndromes, the frequency of the rs12636851C allele was slightly higher in the NAFLD subjects than in the healthy controls ($\chi^2$ test, $P = 0.038$, Table 3). However, the deficiency syndrome subjects exhibited an association between SCAP rs12636851 and NAFLD when the results were stratified for the deficiency syndrome and excess syndrome. Similar associations were not seen in the excess syndrome analysis (Table 3). In the deficiency syndrome group, the subjects who had the CC or TC genotype had a threefold elevated risk for NAFLD compared with the TT genotype (adjusted OR, 3.107; 95% CI, 1.023–9.433, $P = 0.045$; adjusted OR, 2.970; 95% CI, 1.121–7.864, $P = 0.028$). The carriers of the rs12636851C allele had an increased prevalence of NAFLD and an OR of 3.017 (95% CI, 1.208–7.532; $P = 0.018$, when adjusted for age, gender, smoking status, and BMI) compared with the homozygous of the T allele (Table 3).

The observed rs12636851 distributions in the deficiency and excess syndrome groups were shown in Table 4. The ratio of the subjects with the CC genotype and C allele in the deficiency syndrome group were larger than that in the excess syndrome group after the adjustment for age, gender, smoking status, and BMI (OR, 3.120; 95% CI, 1.334–7.297, $P = 0.009$; OR, 2.536; 95% CI, 1.250–5.146, $P = 0.010$).

Discussion

The results of this study provide preliminary evidence for the interlinking of SCAP gene polymorphisms to the TCM syndromes associated with NAFLD. In the present study, SCAP rs12636851 showed a significant genotype and allelic variation between the deficiency syndrome and healthy control subjects as well as between the deficiency and excess syndrome subjects (when the NAFLD subjects were classified according to deficiency and excess syndrome). Results suggested that the deficiency syndrome subjects are genetically susceptible to NAFLD.

Some Chinese herbs have been demonstrated to be successful in treating hyperlipidemia and fatty liver [20–22]. Thus, TCM may be a considerable option for the treatment of NAFLD. An appropriate and effective TCM treatment is based on a valid TCM syndrome classification. However, NAFLD is characterized by clinical heterogeneity, and the consensus on the description of TCM syndromes is low in literature. Therefore, a correspondence between nosology and biomedical background is necessary to ensure the diagnostic stability of the TCM syndromes [23]. Increasing numbers of researchers have explored the biological and molecular basis of TCM syndromes with various methods and achieved remarkable progress [24,25].

In TCM, it is usual to provide different treatments for the same disease based on the patient’s syndrome classification, which
Table 3
Association between SCAP rs12636851 genotypes and NAFLD.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Healthy control (n=100)</th>
<th>NAFLD (n=186)</th>
<th>Adjusted OR(95% CI)</th>
<th>P</th>
<th>( \chi^2 )</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xu pattern + Shi pattern</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>37(37.0)</td>
<td>47(25.3)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>44(44.0)</td>
<td>90(48.4)</td>
<td>1.711(0.822–3.562)</td>
<td>0.151</td>
<td>4.790</td>
<td>0.091</td>
</tr>
<tr>
<td>CC</td>
<td>19(19.0)</td>
<td>49(26.3)</td>
<td>1.700(0.719–4.022)</td>
<td>0.227</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC+CC</td>
<td>63(63.0)</td>
<td>139(74.7)</td>
<td>1.708(0.867–3.362)</td>
<td>0.122</td>
<td>4.385</td>
<td>0.038</td>
</tr>
<tr>
<td>Shi pattern</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>37(37.0)</td>
<td>32(33.7)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>44(44.0)</td>
<td>43(45.3)</td>
<td>0.972(0.411–2.299)</td>
<td>0.948</td>
<td>0.271</td>
<td>0.845</td>
</tr>
<tr>
<td>CC</td>
<td>19(19.0)</td>
<td>20(21.1)</td>
<td>1.061(0.382–2.942)</td>
<td>0.910</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC+CC</td>
<td>63(63.0)</td>
<td>63(66.3)</td>
<td>1.002(0.455–2.207)</td>
<td>0.997</td>
<td>0.234</td>
<td>0.628</td>
</tr>
<tr>
<td>Xu pattern</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>37(37.0)</td>
<td>15(16.5)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>44(44.0)</td>
<td>47(51.6)</td>
<td>2.970(1.121–7.864)</td>
<td>0.028</td>
<td>11.090</td>
<td>0.004</td>
</tr>
<tr>
<td>CC</td>
<td>19(19.0)</td>
<td>29(31.9)</td>
<td>3.107(1.023–9.433)</td>
<td>0.045</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC+CC</td>
<td>63(63.0)</td>
<td>76(83.5)</td>
<td>3.017(1.208–7.532)</td>
<td>0.018</td>
<td>10.122</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 4
Association between SCAP rs12636851 genotypes and TCM pattern classification.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Shi pattern (n=95)</th>
<th>Xu pattern (n=91)</th>
<th>Adjusted OR(95% CI)</th>
<th>P</th>
<th>( \chi^2 )</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>32(33.7)</td>
<td>15(16.5)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>43(45.3)</td>
<td>47(51.6)</td>
<td>2.269(1.071–4.804)</td>
<td>0.032</td>
<td>7.897</td>
<td>0.019</td>
</tr>
<tr>
<td>CC</td>
<td>20(21.1)</td>
<td>29(31.9)</td>
<td>3.120(1.334–7.297)</td>
<td>0.009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC+CC</td>
<td>63(66.3)</td>
<td>76(83.5)</td>
<td>2.536(1.250–5.146)</td>
<td>0.010</td>
<td>7.282</td>
<td>0.007</td>
</tr>
</tbody>
</table>

corresponds to the personalized treatment in western medicine. Moreover, genetic information is used for the diagnosis, early intervention, and personalized treatment of the disease. The apparent overlap in the aim of the TCM syndrome classification and genetic genotype suggests that genetic polymorphisms may provide a scientific evidence for TCM syndrome classification. In the recent years, several studies made efforts to explain the TCM syndromes from the perspective of genetic polymorphisms [26,27]. In the current study, we classified NAFLD into the deficiency and excess syndrome. The statistical analysis demonstrated that SCAP rs12636851 SNP was associated with the deficiency syndrome. Therefore, this association can provide, at least in part, a scientific evidence for the validity of the TCM syndrome classification of NAFLD. In future studies, independent and larger populations are needed to replicate and verify this finding.

A considerable number of studies demonstrated that SCAP has a central function in lipid metabolism, especially in the liver [12,28]. Sterol-sensing function is an important function of SCAP. When cellular cholesterol rises, SCAP senses the excess cholesterol, and change its conformation in such a way that the SCAP/SREBP complex is no longer incorporated into the endoplasmic reticulum (ER) transport vesicles; thus the active fragment cannot reach the nucleus. As a result, the synthesis of cholesterol and fatty acids declines [29]. A number of studies reported that mutations on cholesterol-sensing function of SCAP influence cholesterol sensitivity and feedback [30,31]. Rs12636851 is located in the intron of SCAP. An adequate understanding on the roles of intronic variants is an important prerequisite to unravel the intricate genetic basis of complex diseases [32,33]. Common molecular mechanisms for an intronic SNP to the gene expression are to affect transcription, RNA elongation, splicing, or maturation [32]. The SCAP rs12636851 intronic variant in the NAFLD subjects with deficiency syndrome maybe impact the splicing of nearby exons because rs12636851 is located between the exons 13 and 14, resulting in the structural change of SCAP and attenuation of its sterol-sensing function. Consistent with this explanation, we found that the low-density lipoprotein cholesterol (LDL-c) was slightly higher (P=0.039) in the deficiency syndrome group (Table 1). We speculated that due to the attenuation of the sterol-sensing function of SCAP, SCAP was overproduced, the excess SCAP binding SREBP moved to the Golgi complex, and then increased the synthesis of cholesterol and fatty acids. Cholesterol and fatty acid biosynthesis occurs mainly in the liver, thus, the excessive endogenous cholesterol in liver was delivered to other tissues and cells in LDL through the blood circulation, resulting in the increased LDL-c; meanwhile, excessive fatty acids were accumulated in the liver, resulting in the fatty liver. This explanation is also reasonable from the TCM viewpoint. The decreased cholesterol sensitivity of SCAP can be included into the TCM concept of deficiency, which represents negative, inactive, static, attenuated, inhibited, etc. Thus, such accumulating evidence supports the hypothesis that the SCAP rs12636851 polymorphisms in the deficiency syndrome subjects affects the cholesterol-sensing function of SCAP, increasing cholesterol and fatty acid synthesis in liver. Therefore, future studies are warranted to clarify the mechanism on the effect of SCAP rs12636851 in the RNA splicing of nearby exons by heterogeneous nuclear RNA (hnRNA) analysis.
Conflict

Conclusion

SCAP rs12636851 SNP was positively associated with NAFLD in those deficiency syndrome subjects, but not for those presenting with the excess syndrome. Thus, this SNP may help in understanding the genetic basis of NAFLD patients with deficiency syndrome, and in the development of personalized medical care. Moreover, this SNP can provide a novel target for clarifying the mechanism of TCM treatment for NAFLD.

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eujim.2013.06.003.

References


