RESEARCH NOTE

SCAP gene polymorphisms decrease the risk of nonalcoholic fatty liver disease in females with metabolic syndrome

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is recognized as one of the most common causes of chronic liver disease worldwide. The risk of susceptibility to this disease has been shown to be higher in patients with metabolic syndrome (MS) (Alberti *et al.* 2005). NAFLD is a specific hepatic manifestation of MS (Almeda-Valdes *et al.* 2009), and strongly associated with MS components, in particular, dyslipidaemia. Dyslipidaemia is found in 50–60% of individuals with NAFLD (Assy *et al.* 2000). However, other factors also affect NAFLD development and progression besides dyslipidaemia. Hepatic steatosis indicates net fat retention within the hepatocytes and circulating free fatty acids (FFA). This increase in circulating FFA may enhance fat uptake by the liver, thereby resulting in a fatty liver. NAFLD and dyslipidaemia also share common molecular mediators; therefore, several lipid-lowering agents can effectively improve NAFLD (Chatrath *et al.* 2012).

The pathogenesis of NAFLD is complex and multifactorial, in which environmental and genetic factors interact with each other (Juran and Lazaridis 2006). Although environmental factors that cause NAFLD have been identified (Suzuki *et al.* 2005), the contribution of genetic polymorphisms to disease susceptibility remains unclear. As cholesterol and fatty acid metabolism plays an important function in NAFLD pathogenesis (Wanless and Lentz 1990), genetic variations in candidate genes related to dyslipidaemia susceptibility may be related to NAFLD. Sterol regulatory element-binding protein (SREBP) is one of the major regulators of lipid metabolism (Horton *et al.* 2002). Three isoforms of SREBP are currently known: SREBP-1a, SREBP-1c and SREBP-2. SREBP-1a activates the genes involved in fatty acid and cholesterol synthesis pathways. SREBP-1c is a weaker transcriptional activator than SREBP-1a, but SREBP-1c is the major isoform in the liver. SREBP-1a and SREBP-1c are produced from a single gene called the sterol regulatory element-binding factor-1 (*SREBF-1*). SREBP-2, which is encoded by a separate gene called *SREBF-2*, has an important function in lipid homeostasis (Horton *et al.* 2002). SREBP cleavage activating protein (SCAP) is involved in the maturation of SREBP-1 (both isoforms) and SREBP-2, and transports SREBPs from the endoplasmic reticulum to the Golgi apparatus. The SREBPs are subsequently activated and translocated into the nucleus. The SREBPs bind to SREBP response element (SRE) to stimulate the expression of target genes, which encode enzymes for synthesis and uptake of cholesterol and triglycerides (Matsuda *et al.* 2001). Common variants of the human *SREBF-1*, *SREBF-2* and *SCAP* genes are associated with

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dyslipidaemia (Salek *et al.* 2002; Fiegenbaum *et al.* 2005). Thus, we hypothesized that some variants of these genes may be associated with NAFLD.

A total of 363 unrelated individuals were enrolled in this study to determine the impact of *SCAP* polymorphisms on the risk of NAFLD in individuals with MS. Among 363 individuals enrolled, 100 were healthy control participants and the remaining 263 individuals had been diagnosed with MS. Among the 263 individuals in the MS group, 160 participants had complications due to NAFLD; 103 participants did not have NAFLD. We selected 14 tag single-nucleotide polymorphisms (SNPs) of: *SREBF-1*, *SREBF-2* and *SCAP* genes for analysis in our study. The results showed that rs2101247 SNP of *SCAP* was significantly different between the group with MS and NAFLD (MS + NAFLD) and the group with MS but no NAFLD (MS – NAFLD), but the statistical significance was observed only in females. The variant genotypes of rs2101247 (GA/AA) were associated with a significantly decreased risk for NAFLD (adjusted odds ratios (OR), 0.201; CI, 0.061–0.664; $P = 0.009$; adjusted OR, 0.184; CI, 0.053–0.640; $P = 0.008$) in female participants compared with the *GG* genotype. However, an association between *SCAP* gene polymorphisms and lipid levels/body mass index (BMI) was not found.

Materials and methods

Participants

This study was conducted at Longhua Hospital and Fenglin Community Hospital in the Xuhui District of Shanghai, People's Republic of China, from August 2009 to May 2010. A total of 363 unrelated individuals were enrolled in this study. Of the total number of participants, 100 individuals were randomly selected as healthy control participants with no history of type II diabetes, hypertension, dyslipidaemia and fatty liver, and the remaining 263 individuals had been diagnosed with MS. We performed anthropometric measurements and completed a questionnaire on health-related behaviours and biochemical determinations. MS was defined based on the guidelines for MS proposed by the Chinese Diabetes Society (Li *et al.* 2012). NAFLD was diagnosed according to the guidelines for diagnosis and treatment of NAFLD issued by the Fatty Liver and Alcoholic Liver Disease Study Group of the Chinese Liver Disease Association (2008 and 2010) (Zeng *et al.* 2008). The diagnosis of fatty liver can be made by ultrasonography (Kojima *et al.* 2003).

Selection of tag SNPs

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 (Carlson *et al.* 2004). 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 for three genes were chosen for this study.

Genotyping assays for SNPs

SNPs were typed using iPLEX chemistry on a matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF) (Sequenom, San Diego, USA). Two polymerase chain reactions (PCRs) were conducted in standard 384-well plates. For each reaction, 5 μL solution containing 10 ng of genomic DNA, 0.5 units of *Taq* polymerase (HotStarTaq, Qiagen, Shanghai, China 500 μmol of each deoxynucleotide triphosphate and 100 nmol of each PCR primer. PCR thermal cycling was performed using an ABI-9700 instrument (Applied Biosystems, USA) for 15 min at 94°C, followed by 45 cycles of 20 s at 94°C, 30 s at 56°C and 60 s at 72°C. Following the completion of the PCR, 2 μL (0.3 units) of shrimp alkaline phosphatase (SAP) was added to the reaction. The reaction containing SAP was incubated at 37°C for 20 min. The SAP was then inactivated by incubating the reaction mixture at 85°C for 5 min. After adjusting the concentrations of the extension primers to optimize the signal-to-noise ratio, the post-PCR primer extension reaction of the iPLEX assay was conducted in a final volume of 9 μL, which contained 0.2 μL of the termination mix, 0.04 μL of DNA polymerase (Sequenom) and 625–1250 nmol/L of the extension primers. A two-step 200 short-cycle program was used for the iPLEX reaction. The initial denaturation was performed at 94°C for 30 s and followed by 5 s at 94°C, 5 cycles of 5 s at 52°C and 5 s at 80°C. An additional 40 annealing and extension cycles were then looped back to 5 s at 94°C, 5 cycles of 5 s at 52°C and 5 s at 80°C. The final extension was performed at 72°C for 3 min, and the sample was cooled to 4°C. The samples were then manually desalted using 6 mg of clean resin and a dimple plate. The samples were transferred to a 384-well Spectro-CHIP (Sequenom, San Diego, USA) plate using a nanodispenser. Mass spectrum was acquired using a compact mass spectrometer and analysed by the MassARRAY Typer 4.0 (Sequenom, San Diego, USA) software. The PCR assay was arrayed with two no-template controls and four duplicated samples in each 384-well format as quality controls. All genotyping results were generated and checked by the laboratory staff members who were unaware of the disease status of the patients (Buetow *et al.* 2001).

Statistical analysis

The Hardy–Weinberg equilibrium (HWE) was assessed using a goodness of fit χ^2test. Continuous variables were expressed as mean ± standard deviation (SD), 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 percentages, and then

compared using the χ^2-tests or Fisher's tests. ORs and 95% CI were calculated separately using binary logistic regression analysis adjusted for gender, age, smoking status and BMI. Two-sided P values of <0.05 were considered statistically significant. All statistical analyses were conducted using SPSS ver. 17.0 (SPSS Software, Chicago, USA).

Table 1. Clinical and biological characteristics of subjects in three groups.

	Total		MS	
Character	Healthy control ($n = 100$, 27.5%)	MS ($n = 263$, 72.5%)	With NAFLD ($n = 160$, 60.8%)	Without NAFLD ($n = 103$, 39.2%)
Female (%)	57.0	60.8	65.6	53.4
Age (years)	66.65 ± 5.30	69.87 ± 9.47**	69.65 ± 9.05	70.21 ± 10.12
Smokers (%)	10.0	15.2	13.1	18.4
BMI (kg/m^2)	22.82 ± 1.54	25.51 ± 3.02**	26.73 ± 2.98	23.61 ± 1.92$^{\#\#}$
FPG (mmol/L)	5.13 ± 0.81	7.48 ± 2.19**	7.51 ± 2.20	7.44 ± 2.18
SBP (mm Hg)	128.74 ± 6.54	137.92 ± 15.38**	139.09 ± 16.00	136.10 ± 14.25
DBP (mm Hg)	75.24 ± 5.89	78.90 ± 9.81**	79.68 ± 9.68	77.71 ± 9.93
TG (mmol/L)	1.14 ± 0.33	1.60 ± 1.00**	1.78 ± 1.07	1.31 ± 0.82$^{\#\#}$
TC (mmol/L)	5.31 ± 0.97	4.73 ± 0.73**	5.27 ± 0.88	5.37 ± 1.11
HDL-c (mmol/L)	1.45 ± 0.37	1.30 ± 0.33**	1.29 ± 0.35	1.30 ± 0.30
LDL-c (mmol/L)	2.93 ± 0.74	3.21 ± 0.96*	3.26 ± 0.94	3.13 ± 0.99
VLDL (mmol/L)	2.48 ± 0.54	2.54 ± 0.59	2.54 ± 0.61	2.54 ± 0.56
ALT (U/L)	25.33 ± 13.2	25.30 ± 13.0	26.83 ± 14.16	22.92 ± 10.37$^{\#}$
AST (U/L)	19.24 ± 5.47	20.57 ± 6.93	19.16 ± 6.45	21.49 ± 7.10$^{\#}$

*$P < 0.05$ and **$P < 0.01$ vs the healthy control group, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs the MS-NAFLD group.
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, very-low-density lipoprotein cholesterol; ALT, alanine transaminase; AST, aspartate transaminase.

Table 2. Conditional logistic regression analysis assuming additive and dominant model between MS−NAFLD group and MS+NAFLD group.

	Adjusted OR, 95% CI, P		
SNP	Dominant model	Additive model	χ^2, P
SREBF1			
4925115	1.061, 0.656–1.717, 0.809	0.856, 0.447–1.637, 0.638	3.895, 0.141
8066560	1.183, 0.714–1.962, 0.514	1.001, 0.543–1.844, 0.997	1.853, 0.383
2282180	1.098, 0.637–1.893, 0.737	1.102, 0.594–2.046, 0.758	0.322, 0.839
9902941	1.080, 0.661–1.765, 0.757	1.080, 0.661–1.756, 0.757	2.114, 0.342
SREBF2			
2228314	0.872, 0.491–1.550, 0.641	0.856, 0.459–1.597, 0.625	0.258, 0.927
5996080	1.326, 0.603–2.918, 0.483	1.484, 0.642–3.432, 0.356	1.515, 0.571
2267438	0.941, 0.583–1.519, 0.804	0.541, 0.247–1.184, 0.124	4.120, 0.129
9607852	0.501, 0.125–2.007, 0.329	0.501, 0.125–2.007, 0.329	0.280, 0.772
4822062	0.515, 0.221–1.200, 0.124	0.483, 0.198–1.176, 0.109	3.071, 0.187
17379759	1.062, 0.681–1.655, 0.791	1.050, 0.541–2.038, 0.885	1.689, 0.458
SCAP			
2101247	**0.566, 0.371–0.862, 0.008**	**0.301, 0.140–0.648, 0.002**	**11.101, 0.004**
2306628	1.699, 0.639–4.513, 0.288	1.698, 0.638–4.519, 0.289	0.840, 0.803
4858889	1.162, 0.618–2.188, 0.641	1.169, 0.579–2.360, 0.663	0.696, 0.802
17079634	0.940, 0.509–1.736, 0.843	0.913, 0.465–1.791, 0.791	1.253, 0.554

Adjusted OR: adjusted for age, gender, smoking status, BMI.
Additive model: common homozygotes vs heterozygotes vs rare homozygotes.
Dominant model: common homozygotes vs combined heterozygous and rare homozygous. Only *SCAP* 2101247 is associated with NAFLD is in bold.

Table 3. Association between *SCAP* rs2101247 genotypes and NAFLD.

SNP	MS (*n*, %)		Adjusted OR (95% CI)	*P*
	With NAFLD	Without NAFLD		
Male and female				
GG	51 (31.9)	14 (13.7)	1	
GA	67 (41.9)	56 (54.9)	0.311 (0.138–0.700)	0.005
AA	42 (26.2)	32 (31.4)	0.287 (0.120–0.688)	0.005
GA + AA	109 (68.1)	88 (86.3)	0.301 (0.140–0.648)	0.002
Male				
GG	16 (29.1)	9 (18.8)	1	
GA	27 (49.1)	26 (54.2)	0.441 (0.137–1.418)	0.170
AA	12 (21.8)	13 (27.1)	0.329 (0.116–1.585)	0.204
GA + AA	39 (70.9)	39 (81.2)	0.437 (0.145–1.314)	0.140
Female				
GG	35 (33.3)	5 (9.3)	1	
GA	40 (38.1)	30 (55.6)	0.201 (0.061–0.664)	0.009
AA	30 (28.6)	19 (35.2)	0.184 (0.053–0.640)	0.008
GA + AA	70 (66.7)	49 (90.7)	0.194 (0.062–0.602)	0.005

Results

All the SNPs were in HWE ($P > 0.05$) in the healthy control group. The clinical and laboratory characteristics of the three groups are listed in table 1. In the MS group, triglycerides (TG) and BMI were significantly ($P < 0.001$) higher in the MS + NAFLD group. The relationship between the phenotypes and 14 SNPs in the 363 unrelated participants are shown in table 2; tables 1 and 2 in electronic supplementary material at http://www.ias.ac.in/jgenet/. Only the rs2101247 SNP of *SCAP* was found to be significantly associated with NAFLD in the MS groups in genotypic distributions ($P = 0.004$, table 2), additive model ($P = 0.008$, table 2) and dominant mode ($P = 0.002$, table 2).

In the MS population, *A* allele carriers (GA and AA) of rs2101247 were protected against NAFLD compared to the carriers of homozygous *G* allele of rs2101247 (table 3). The results were stratified by gender, and the female subgroup showed that *SCAP* rs2101247 was associated with NAFLD, but a similar relationship was not observed in the male subgroup. The *A* allele (GA and AA) was associated with a significantly reduced risk of NAFLD in the female participants (adjusted OR, 0.194; CI, 0.062–0.602; $P = 0.005$; table 3). The variant genotypes of rs2101247 (GA/AA) were associated with a significantly decreased risk for NAFLD in the female participants (adjusted OR, 0.201; CI, 0.061–0.664; $P = 0.009$; adjusted OR, 0.184; CI, 0.053–0.640; $P = 0.008$; table 3) compared with the *GG* genotype.

To determine whether the relationship between *SCAP* rs2101247 and the risk of NAFLD was mediated by its effect on plasma lipid profiles or BMI, we observed the clinical and laboratory characteristics of female participants with *GA*, *AA* and *GG* genotypes. No significant differences were found in the clinical and laboratory characteristics of the three groups (table 3 in electronic supplementary material).

Discussion

To the best of our knowledge, this study is the first to demonstrate a relationship between *SCAP* rs2101247 SNP and NAFLD in a MS population. Our results showed that the *SCAP* rs2101247 *A* allele (*AA* and *GA* genotypes) might decrease the risk of NAFLD in female MS population independently from other potential confounding factors.

The development of complex diseases is dependent on many factors. Risk factors for NAFLD include obesity, diabetes, insulin resistance and hypertriglyceridemia (Brunt 2010), all of which are MS components. Epidemiological evidence supported the relationship between NAFLD and MS. In a previously published report, the prevalence of NAFLD among MS participants and non-MS participants was 56.16–70.08% and 12.97–15.35%, respectively (Chen *et al.* 2011), which is consistent with our research on prevalence of NAFLD among MS participants (60.8%) (table 1). Therefore, NAFLD was found to be strongly associated with MS. So, we focussed our research on the development of NAFLD in the population with MS. Genetic factors are also important for the development of NAFLD (Hjelkrem *et al.* 2008). The gene polymorphisms have been shown to bear a positive or a negative correlation with susceptibility to NAFLD in previous studies (Zhou *et al.* 2010). This study demonstrates that rs2101247 polymorphism of *SCAP* protects MS participants from NAFLD.

SCAP has an important function in lipid metabolism, and SCAP deficiency inactivates SREBP-1 and SREBP-2 (Matsuda *et al.* 2001; Moon *et al.* 2012). The effect of a mutant version of *SCAP* expressed in the liver was investigated in transgenic mice. The result showed that levels of nSREBP-1 and nSREBP-2 were increased because of constitutive processing of SREBP. nSREBP-1 and nSREBP-2 increased the expression of SREBP target genes, which

stimulated cholesterol and fatty acid synthesis and caused a significant accumulation of hepatic cholesterol and triglycerides (Korn *et al.* 1998). Other evidence has shown that Cre-mediated *SCAP* disruption significantly reduced nSREBP-1 and nSREBP-2 levels in the liver and decreased SREBP target gene expression in liver-specific *SCAP* knockout mice. As a result, the synthesis rates of cholesterol and fatty acids decreased by 70–80% in *SCAP*-deficient livers (Fan *et al.* 2001; Horton *et al.* 2002). Therefore, SCAP plays a crucial role in the pathogenesis of fatty liver by regulating the accumulation of hepatic cholesterol and triglycerides. Thus, defining the relationship between *SCAP* polymorphisms and the development of NAFLD should be of great value.

The most interesting observation of our study was that *SCAP* polymorphisms protected the participants against NAFLD in the female MS population. This result is consistent with other polymorphism studies on NAFLD, but the reason and mechanisms for the gender-specific difference in genetic susceptibility to NAFLD development are unclear (Song *et al.* 2005; Zhou *et al.* 2010). Interestingly, polymorphisms of *Leptin* -2548 or *PEMT* gene (V175M) are associated with increased susceptibility to NAFLD in women, which were contradictory to our finding. At present, there are no other reports on *SCAP* polymorphism rs2101247. We cannot exclude the possibility that the gender difference observed in our study for *SCAP* polymorphism rs2101247 is caused by false negatives. As sample size was relatively small for genotype distributions in the male subgroup, the analysis might be prone to false negatives due to low statistical power. Therefore, this finding should be validated in larger and more diverse cohorts.

In our study, the association between *SCAP* gene polymorphisms and lipid levels/BMI was not observed. BMI and lipid levels were strongly related to NAFLD but did not completely indicate the status of NAFLD. Although NAFLD is usually associated with obesity and hyperlipidaemia, nonobese patients can also present NAFLD (lean NAFLD). In fact, it has been observed that 25% of the patients with NAFLD are not obese and/or have a normal lipidemic profile, and this finding is particularly true for Asian populations (Pagadala and McCullough 2012). Thus, the distribution of body fat (not total fat) and especially, the liver fat were associated with NAFLD.

Therefore, future studies should focus on investigating the expression level of *SCAP* in the livers of patients with NAFLD and between the patients with or without *A* allele. In addition, the mechanism by which *SCAP* polymorphism protects individuals with MS from developing NAFLD needs further investigation.

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