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

Shanshan Sun, Tao Wu, Miao Wang, Wei Li, Lin Wang, Songhua He, Huafeng Wei, Haiyan Song, Guang Ji.

Shanghai University of Traditional Chinese Medicine, China

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OUTLINE

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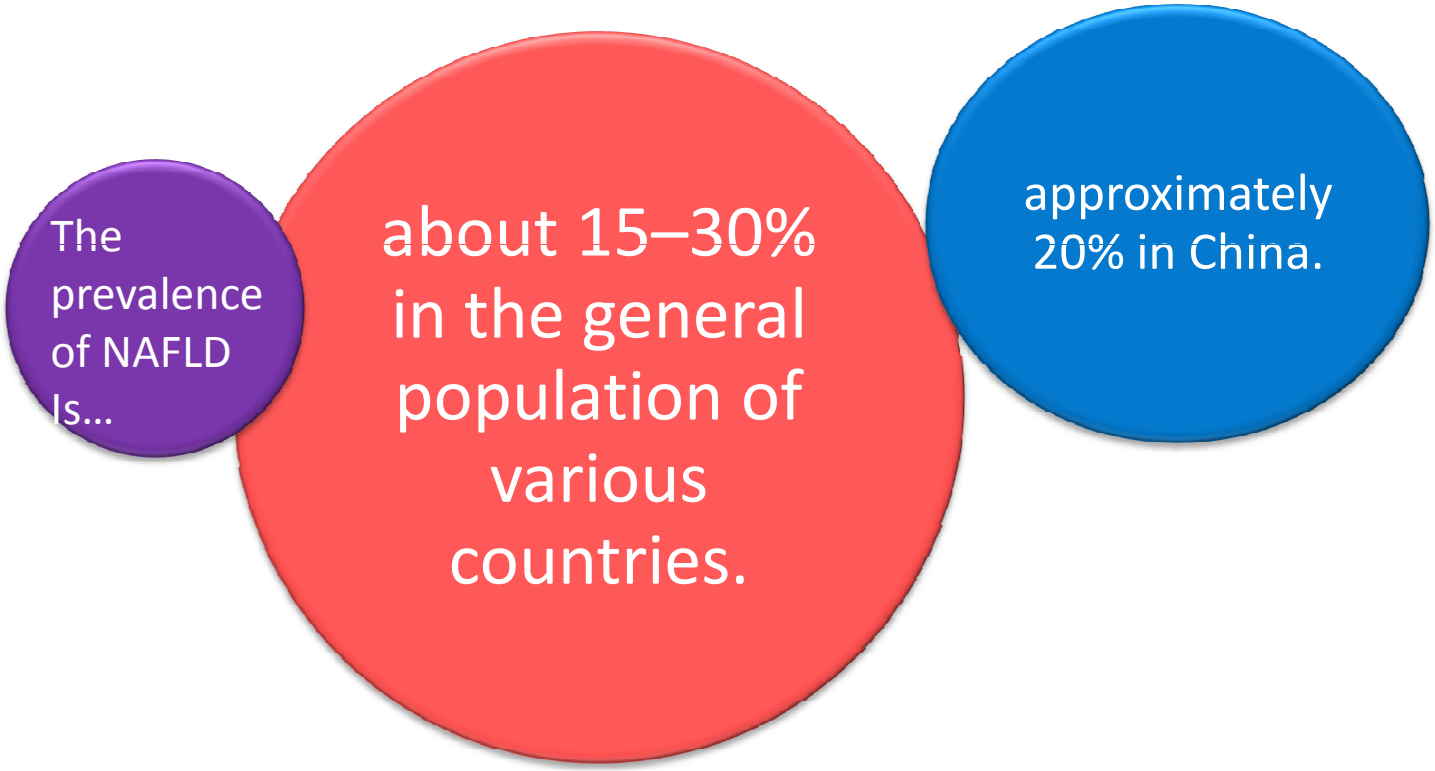
Background

Epidemiology

Non-alcoholic fatty liver disease (NAFLD) is recognized as one of the most common causes of chronic liver disease worldwide.

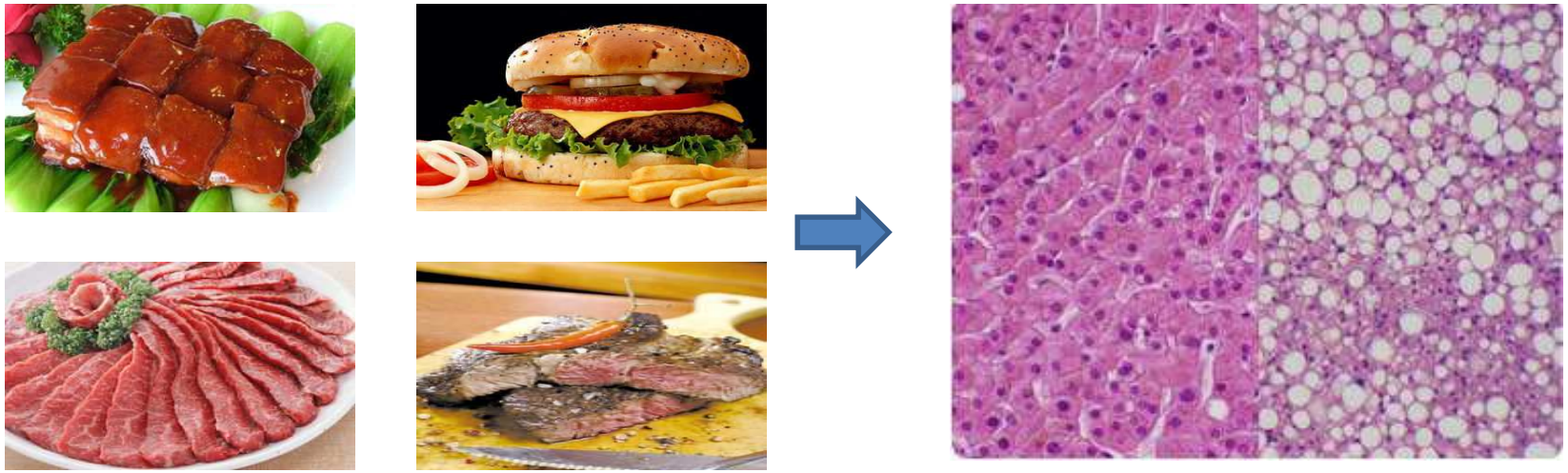


TCM application in NAFLD therapy

- 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 . This method of classifications limits the clinical heterogeneity of NAFLD and provides a basis for developing a classified treatment protocol.

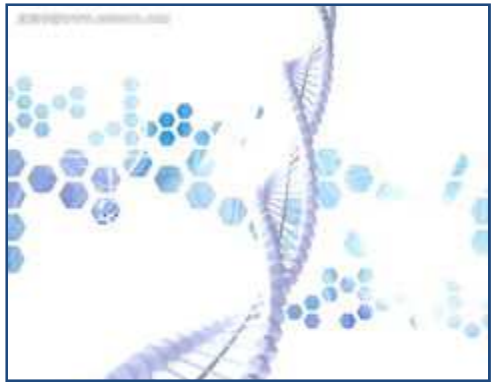
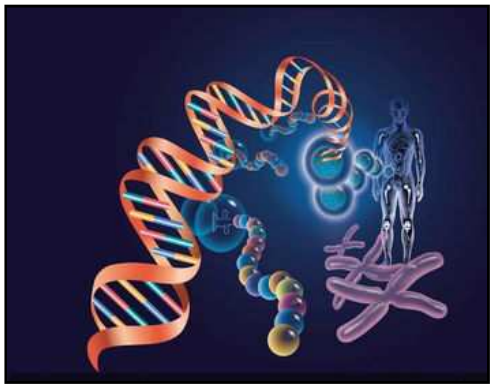
The pathogenesis of NAFLD

- The pathogenesis of NAFLD is complex and multifactorial, as environmental and genetic factors interact with each other.
- Environmental factors such as excessive calorie intake and a lack of daily physical activity are undoubtedly fuelling the epidemic of NAFLD. However, environmental factors are not solely responsible for the NAFLD problem.



The pathogenesis of NAFLD

- 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.

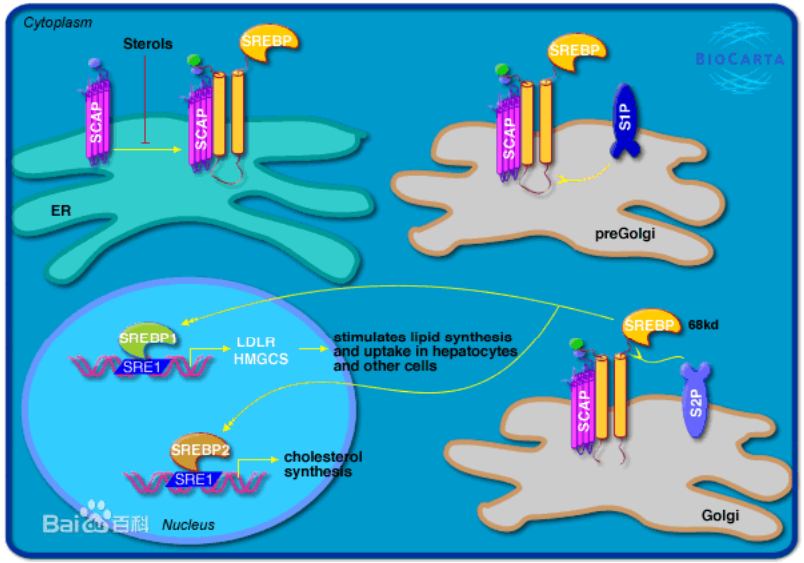




Genes associated with NAFLD

- In the recent years, several genes have been suggested potentially associated with NAFLD-related traits in the general population, such as TLR4, PPAR, Glucokinase regulatory protein (GCKR) and etc.
- However, the contribution of genetic polymorphisms to the disease susceptibility is still inconclusive.

Genes associated with NAFLD

- As cholesterol and fatty acid metabolism plays an important role in NAFLD pathogenesis, genetic variations in candidate genes related to dyslipidemia susceptibility may be associated with NAFLD.



- Sterol regulatory element binding proteins (SREBPs) are known to function as transcription factors that activate specific genes involved in cholesterol and fatty acid metabolism . SREBPs are produced from separated genes named sterol regulatory element-binding factors-1 (SREBF-1) and SREBF-2.
- The SREBP cleavage activating protein (SCAP) is involved in maturation of both SREBPs and transports SREBPs from the endoplasmic reticulum to the Golgi complex. The SREBPs are subsequently activated and translocated into the nucleus.

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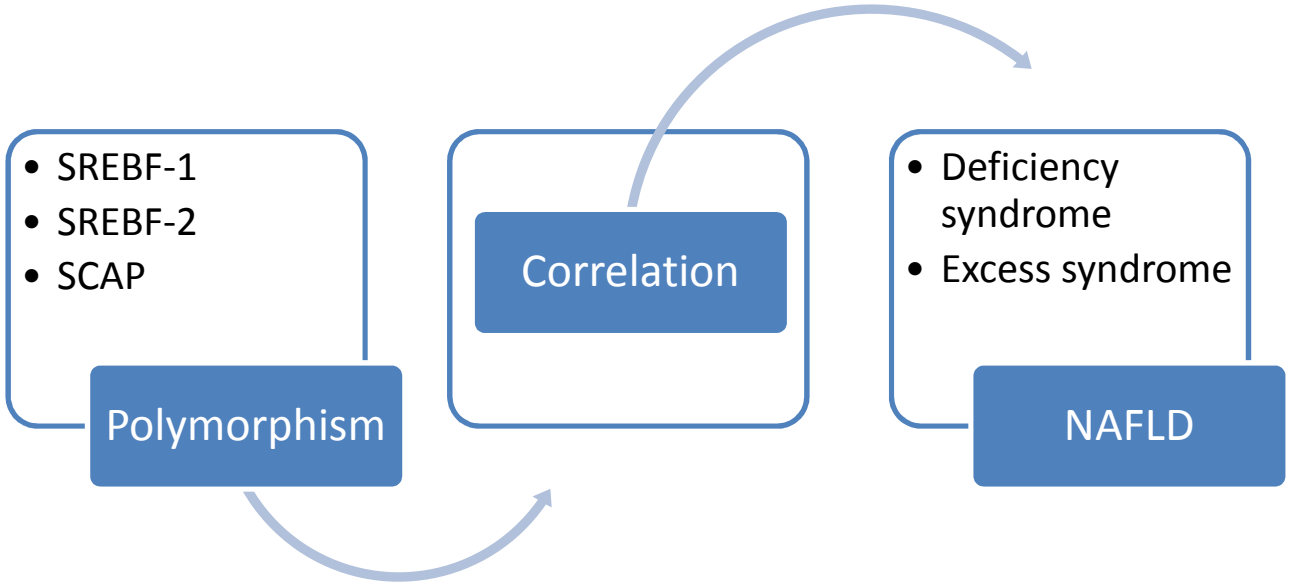
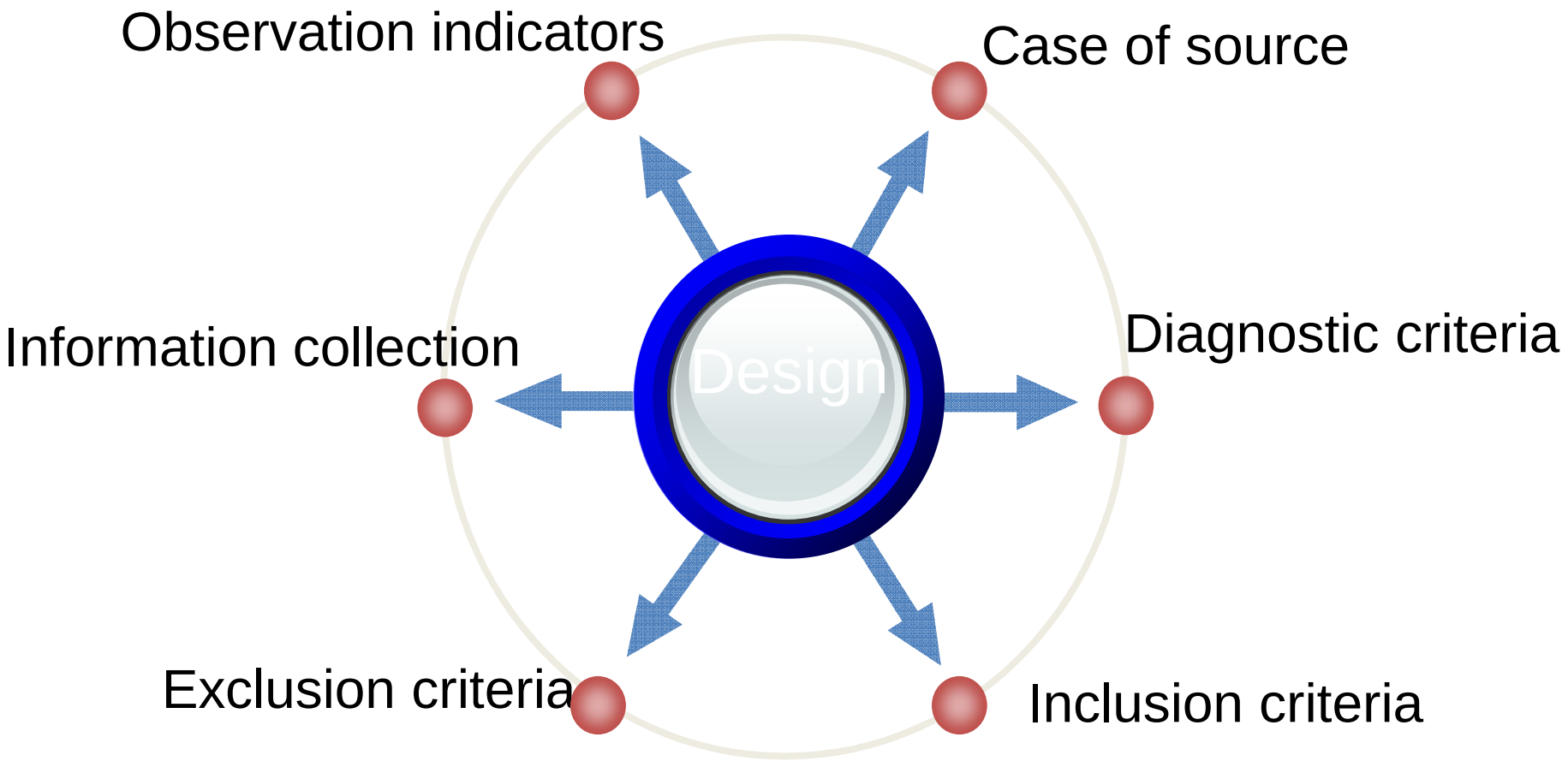
Objective

- In the current study, we investigated whether the single nucleotide polymorphisms (SNPs) are of SREBF-1, SREBF-2, and SCAP genes associated with the TCM syndromes of NAFLD.



Method

Project Design



Subjects

- Longhua Hospital, Shanghai University of TCM and Fenglin Community Hospital in the Xuhui District of Shanghai, from August 2009 to May 2010.
- 211 individuals were diagnosed with NAFLD, and the remaining 100 individuals were selected as healthy control subjects with no history of fatty liver.

Diagnostic criteria---NAFLD

NAFLD was diagnosed according to the guidelines issued by the Fatty Liver and Alcoholic Liver Disease Study Group of the Chinese Liver Disease Association (2010).

I.There is no history of drinking alcohol, or ethanol intake per week is less than 140 g in men and 70 g in women.

II.Specific diseases that can result in fatty liver, such as viral hepatitis, drug-induced liver disease, total parenteral nutrition, and Wilson's disease can be ruled out.

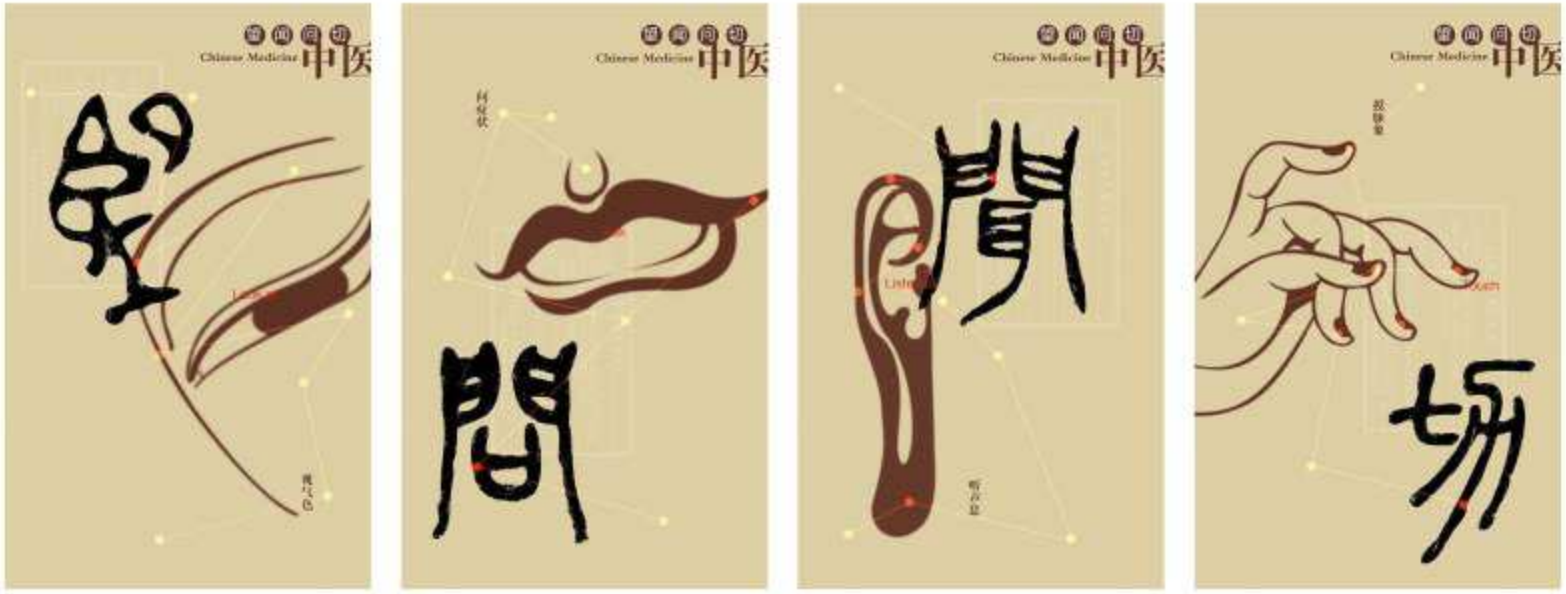
III.The result of the liver imaging study meets the imaging diagnostic criteria of diffused fatty liver with unknown causes, and/or

IV.metabolic syndrome constituents, such as overweight, hyperglycemia, blood lipid disorder, and hypertension occur, with an unexplained increase in serum levels of ALT and/or AST and γ-GT.

V.Fatty liver can be diagnosed by ultrasonography when the findings present the following: stronger liver echogenicity than kidney or spleen, deep attenuation of ultrasound signal, and vascular blurring and narrowing of the hepatic vein lumen. (J Gastroenterol. 2003;38:954-961.)

Diagnostic criteria---TCM syndromes Differentiation

- The differentiation of deficiency syndrome and excess syndrome of the TCM theory is based on “Textbooks for general tertiary education of Chinese medicine: diagnosis of Chinese medicine”(Ministry of Health of China)



Flow diagram

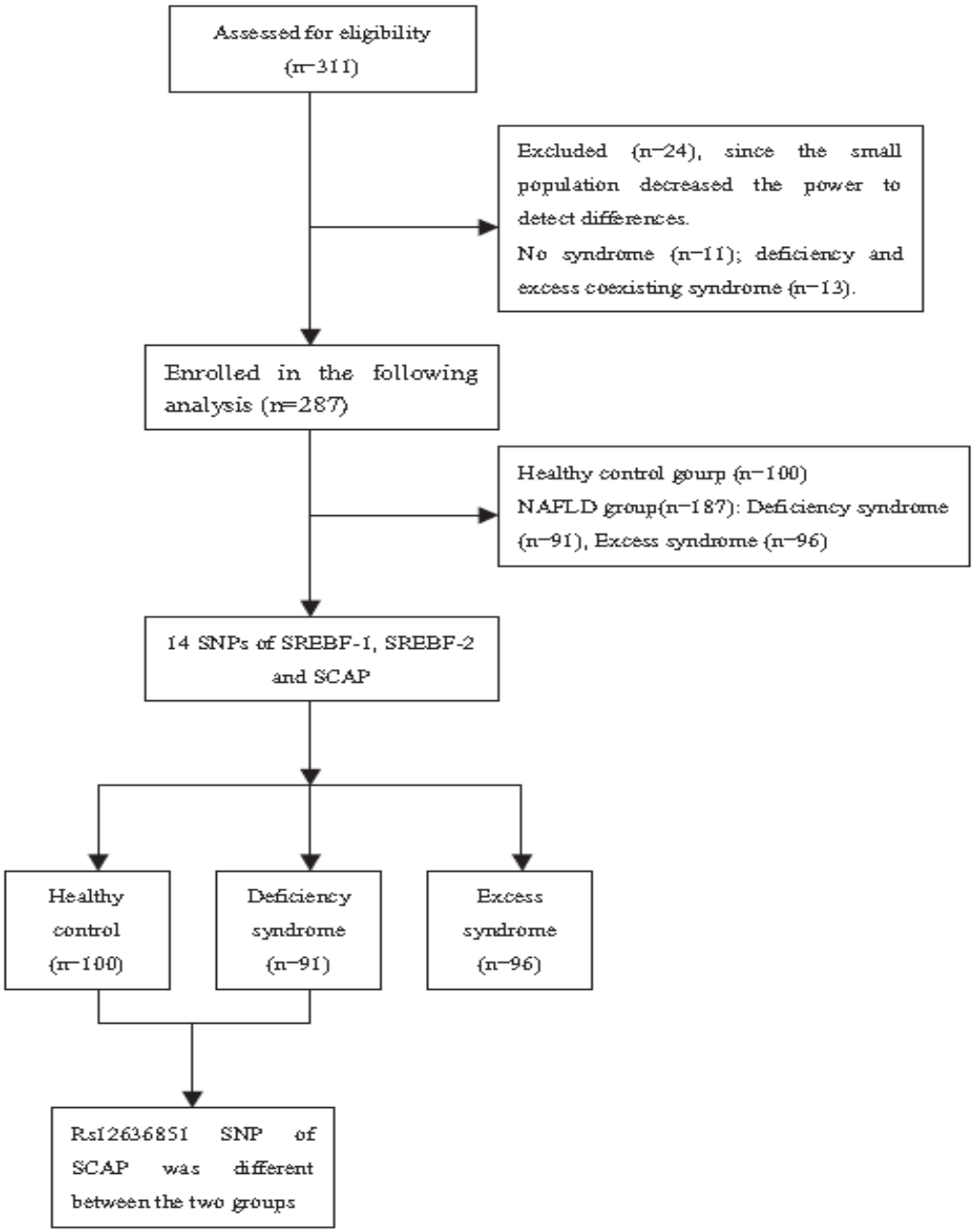
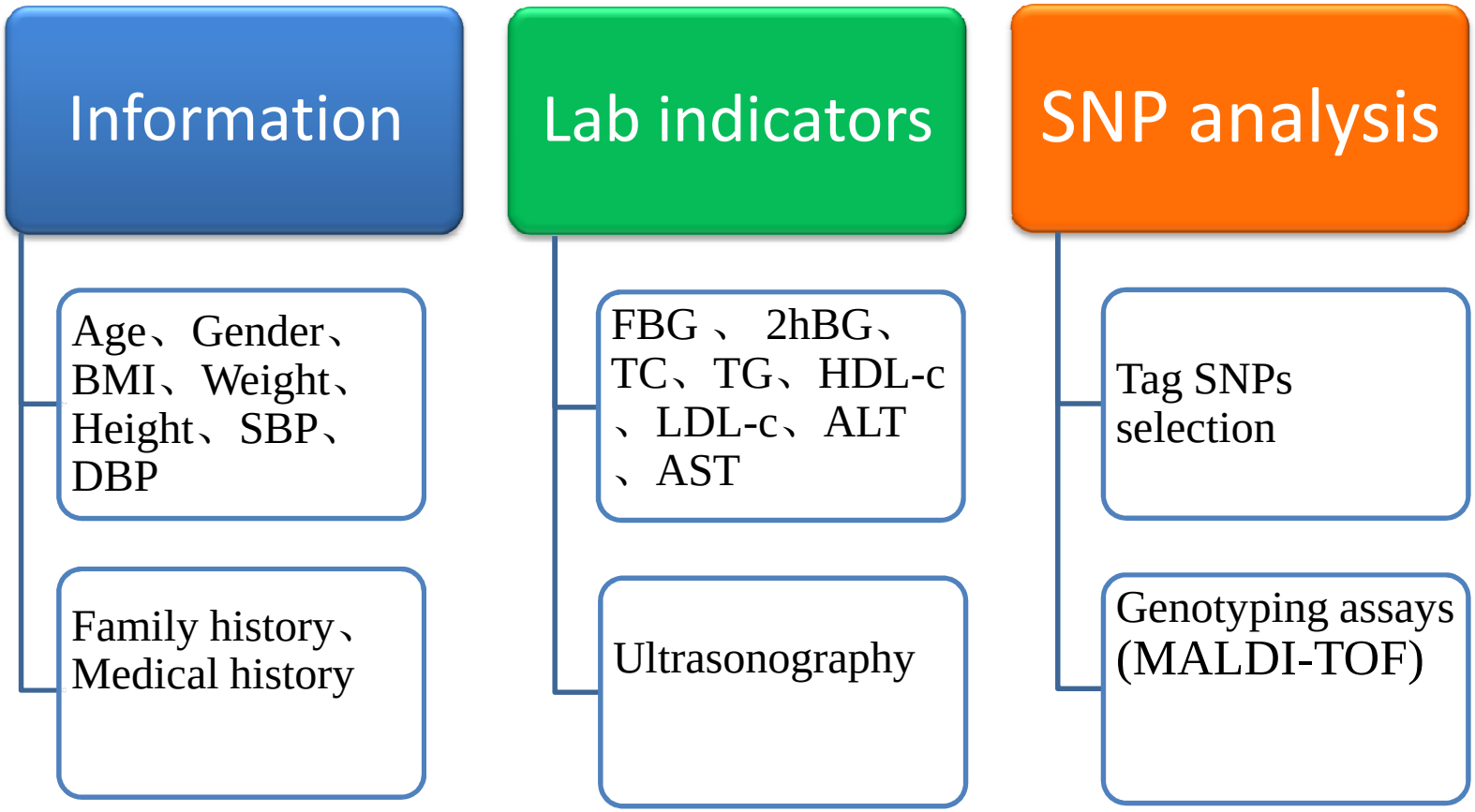


Fig. 1. Flow diagram.

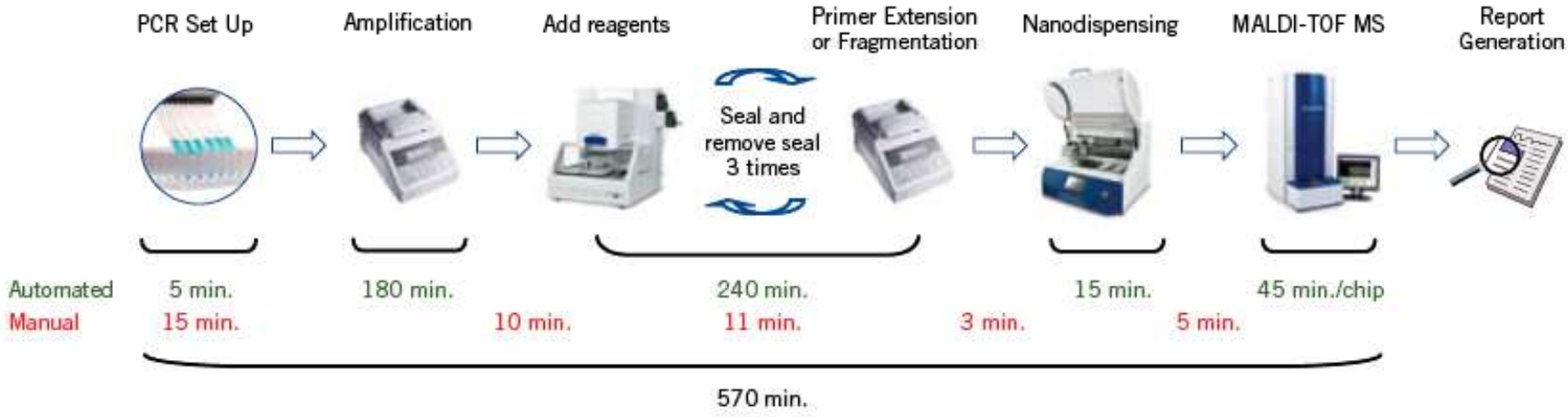
Clinical and laboratory evaluation



SNP genotyping assays

- 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.

- SNPs were typed using iPLEX chemistry on a matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOFMS).



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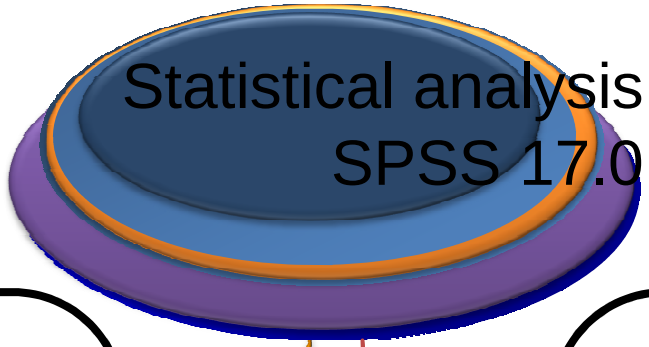
Statistical analysis



- Continuous variables --- Student's *t*-test or Kruskal–Wallis test.
- Categorical variables --- the χ^2-test or Fisher's test.
- The Hardy–Weinberg equilibrium ---goodness-of-fit χ^2-tests
- α=0.05

- Odds ratios (ORs) and 95% confidence interval (CI) --- binary logistic regression analysis
- An additive model :

0---AA (major homozygous),
1---Aa (heterozygous),
2---aa (minor homozygous)

- A dominant model :

0---AA (major homozygous) ,
1--- Aa + aa (heterozygous combined with minor homozygous)

Results

3.1 The clinical and laboratory characteristics of the four groups

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

Characteristics	Total		NAFLD	
	NAFLD ($n=187$)	Healthy control ($n=100$)	Deficiency syndrome (n=91)	Excess syndrome (n=96)
Female (%)	62.6	57.0	63.7	61.5
Age (years)	69.96 ± 8.70	66.65 ± 5.30*	70.89 ± 8.95	69.08 ± 8.42
Smokers (%)	13.9	10.0	14.3	13.5
BMI (kg/m^2)	26.07 ± 2.89	22.82 ± 1.54**	25.92 ± 2.56	26.21 ± 2.82
FPG (mmol/L)	7.51 ± 2.29	5.13 ± 0.81**	7.46 ± 2.28	7.57 ± 2.3
SBP (mmHg)	138.01 ± 15.46	128.74 ± 6.54**	137.85 ± 15.02	138.17 ± 15.93
DBP (mmHg)	79.38 ± 9.74	75.24 ± 5.89**	78.88 ± 9.46	79.85 ± 10.02
TG (mmol/L)	1.73 ± 1.10	1.14 ± 0.33**	1.73 ± 0.98	1.67 ± 1.04
TC (mmol/L)	5.32 ± 0.96	4.73 ± 0.73**	5.43 ± 0.95	5.21 ± 0.95
HDL-c (mmol/L)	1.28 ± 0.34	1.45 ± 0.37**	1.32 ± 0.42	1.24 ± 0.24
LDL-c (mmol/L)	3.19 ± 0.97	2.93 ± 0.74*	**3.33 ± 0.91**	**3.04 ± 1.00*****
VLDL (mmol/L)	2.54 ± 0.61	2.48 ± 0.54	2. 61 ± 0.60	2.48 ± 0.60
ALT (U/L)	26.79 ± 13.6	25.33 ± 13.2	27.02 ± 13.81	26.58 ± 13.41
AST (U/L)	20.86 ± 7.54	19.30 ± 5.44	20.53 ± 7.38	21.17 ± 7.71

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 excess syndrome group.

3.2 Conditional logistic regression analysis

Table 2
Conditional logistic regression analysis assuming additive and dominant model between deficiency syndrome group and healthy control group.

SNP	Genotype call number	Major allele/minor allele	Adjusted OR, 95% CI, *P*		χ^2, *P*	P_{hwe}control
			(Dominant model)	(Additive model)		
SREBF1						
4925115	277	A/G	1.473, 0.659–3.291, 0.346	1.492, 0.766–2.906, 0.240	1.170, 0.555	0.705
8066560	286	A/G	1.575, 0.717–3.459, 0.258	1.573, 0.814–3.041, 0.178	2.725, 0.250	0.804
2282180	286	G/A	0.641, 0.285–1.442, 0.282	0.645, 0.314–1.326, 0.233	1.356, 0.509	0.871
9902941	285	C/T	1.450, 0.661–3.178, 0.354	1.488, 0.773–2.864, 0.234	3.374, 0.184	0.911
SREBF2						
2228314	286	G/C	0.527, 0.233–1.191, 0.124	0.608, 0.306–1.211, 0.157	3.638, 0.158	0.711
5996080	286	T/C	0.535, 0.194–1.470, 0.225	0.626, 0.252–1.553, 0.312	2.311, 0.272	0.708
2267438	276	T/C	1.442, 0.597–3.484, 0.416	1.357, 0.742–2.482, 0.321	1.112, 0.580	0.022
9607852	287	G/A	3.141, 0.462–21.373, 0.242	3.141, 0.462–21.373, 0.242	1.370, 0.314	0.846
4822062	287	G/A	0.887, 0.281–2.200, 0.838	0.873, 0.284–2.686, 0.812	1.228, 0.674	0.858
17379759	286	A/G	0.322, 0.051–2.014, 0.226	0.322, 0.051–2.014, 0.226	0.720, 0.443	0.894
SCAP						
12636851	**286**	**C/T**	**3.017, 1.208–7.532, 0.018**	**1.767, 1.022–3.054, 0.041**	**11.090, 0.004**	0.365
2306628	287	C/T	1.998, 0.647–6.170, 0.229	1.998, 0.647–6.170, 0.229	1.257, 0.283	0.883
4858889	270	A/G	1.128, 0.459–2.773, 0.793	1.086, 0.498–2.367, 0.836	1.199, 0.532	1.000
17079634	287	T/C	1.306, 0.538–3.170, 0.555	1.201, 0.550–2.621, 0.646	0.316, 0.868	0.848

Adjusted OR = adjusted for age, gender, smoking status, BMI.
The mean genotype call rate was 98.8%.

Suptable 3: Conditional logistic regression analysis assuming additive and dominant model between excess syndrome group and healthy control group

SNP	adjusted OR, 95%CI, *P*		$\chi 2$, *P*
	dominant model	additive model	
SREBF1			
4925115	1.332,0.735-2.414,0.344	1.174,0.539-2.559,0.686	3.536, 0.171
8066560	1.134,0.600-2.145,0.699	1.087,0.497-2.476,0.834	1.188, 0.548
2282180	0.993,0.510-1.935,0.985	0.934,0.431-2.028,0.864	1.105, 0.645
9902941	1.091,0.580-2.051,0.787	1.026,0.473-2.223,0.949	1.193, 0.591
SREBF2			
2228314	0.662,0.333-1.318,0.240	0.752,0.351-1.612,0.463	3.066, 0.223
5996080	0.757,0.331-1.734,0.510	0.810,0.339-1.937,0.636	0.918,1.000
2267438	1.418,0.804-2.502,0.228	1.075,0.467-2.474,0.866	2.285,0.327
9607852	0.581,0.027-12.472,0.728	0.581,0.027-12.472,0.728	0.003,1.000
4822062	0.802,0.280-2.291,0.680	0.805,0.274-2.364,0.693	0.508,0.840
17379759	1.112,0.274-4.524,0.882	1.107,0.269-4.555,0.888	1.390,0.532
SCAP			
12636851	1.024,0.618-1.696,0.927	1.002,0.455-2.207,0.997	0.271,0.845
2306628	0.641,0.172-2.397,0.509	0.640,0.171-2.389,0.508	1.840,0.437
4858889	1.231,0.577-2.629,0.591	1.366,0.588-3.172,0.468	0.363,0.945
17079634	1.077,0.498-2.330,0.850	1.170,0.494-2.771,0.721	0.443,0.947

adjusted OR=adjusted for age, gender, smoking status, BMI

additive model = common homozygotes versus heterozygotes versus rare homozygotes

dominant model= common homozygotes versus combined the heterozygous and rare homozygous

Suptable 4: Conditional logistic regression analysis assuming additive and dominant model between NAFLD group and healthy control group

SNP	adjusted OR, 95%CI, *P*		$\chi 2$, *P*
	dominant model	additive model	
SREBF1			
4925115	1.009,0.628-1.622,0.969	1.192,0.624-2.276,0.595	0.946, 0.627
8066560	1.225,0.760-1.973,0.405	1.381,0.757-2.519,0.292	0.951, 0.640
2282180	0.945,0.548-1.631,0.839	0.956,0.516-1.771,0.886	0.232, 1.000
9902941	1.287,0.808-2.047,0.288	1.377,0.756-2.506,0.296	0.948, 0.641
SREBF2			
2228314	0.636,0.361-1.119,0.117	0.579,0.312-1.073,0.083	3.520 0.215
5996080	0.730,0.341-1.564,0.418	0.648,0.290-1.447,0.289	2.855,0.198
2267438	0.947,0.615-1.459,0.805	1.265,0.639-2.504,0.500	2.347,0.313
9607852	2.276,0.539-9.601,0.263	2.276,0.539-9.601,0.263	1.227,0.321
4822062	0.845,0.371-1.927,0.689	0.907,0.378-2.177,0.828	0.922,1.000
17379759	0.775,0.274-2.193,0.632	0.861,0.276-2.689,0.796	0.983,1.000
SCAP			
12636851	1.708,0.867-3.362,0.122	1.114,0.767-1.619,0.570	4.790,0.091
2306628	1.953,0.789-4.837,0.148	2.417,0.910-6.421,0.077	4.815,0.058
4858889	1.039,0.574-1.879,0.900	0.901,0.491-1.799,0.768	2.064,0.370
17079634	1.232,0.671-2.260,0.501	1.179,0.593-2.345,0.639	1.157,0.588

3.3 Association between SCAP rs12636851 genotypes and NAFLD

Table 3
Association between SCAP rs12636851 genotypes and NAFLD.

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

3.4 Association between SCAP rs12636851 genotypes and TCM pattern classification

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

SNP	Excess syndrome (n=96)	Deficiency syndrome (n=91)	Adjusted OR(95% CI)	*P*	χ^2	*P*
TT	32(33.7)	15(16.5)	1			
TC	43(45.3)	47(51.6)	**2.269(1.071–4.804)**	**0.032**	**7.897**	**0.019**
CC	20(21.1)	29(31.9)	**3.120(1.334–7.297)**	**0.009**		
TC + CC	63(66.3)	76(83.5)	**2.536(1.250–5.146)**	**0.010**	**7.282**	**0.007**

- 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).

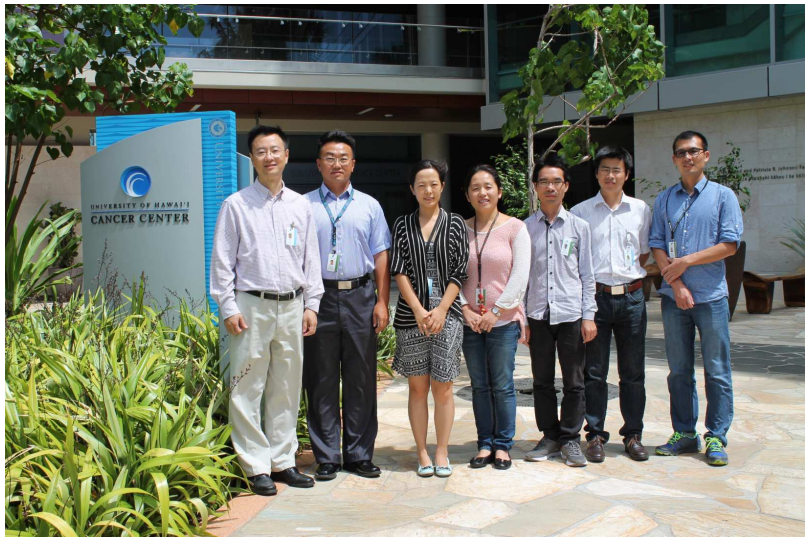
Conclusion

- The results of this study provide preliminary evidence for the interlinking of SCAP gene polymorphisms to the TCM syndromes associated with NAFLD.

- 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.

- 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, it can provide a novel target for clarifying the mechanism of TCM treatment for NAFLD.

Acknowledgement



Prof. Guang Ji

Dr. Tao Liu
Dr. Shanshan Sun
Dr. Yang Ming
Dr. Haiyan Song

Prof. Wei Jia

Dr. Guoxiang Xie
Dr. Xiaoning Wang
Dr. Yan Ni
Dr. Kejun Zhou

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.

- 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. Using the Haploview 4.2 program (http://www.broad.mit.edu/haploview), 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.