

SREBP-2 1784 G/C genotype is associated with non-alcoholic fatty liver disease in north Indians

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Abstract. *Background:* Genetics of non-alcoholic fatty liver (NAFLD) in Asian Indians has been inadequately investigated. This study aims to determine the association of the 1784G > C polymorphism in the *SREBP-2* gene with NAFLD in Asian Indians in north India.

Methods: In this study, ($n = 335$); 162 obese with NAFLD, 91 obese without NAFLD and 82 non-obese without NAFLD subjects were recruited. Abdominal ultrasound, clinical profile, anthropometry, metabolic profile, serum levels of alanine aminotransferase, aspartate aminotransferase, fasting insulin and high sensitivity C-reactive protein (hs-CRP) were analysed. Polymerase chain reaction and restriction fragment length polymorphism were used to identify individual genotypes, and the association of this polymorphism with clinical and biochemical parameters was assessed.

Results: The observed frequency of G allele was 0.73 and C allele was 0.27. Frequency of C/C genotype was higher in NAFLD as compared to obese and non-obese subjects ($p = 0.003$). In NAFLD subjects 57.4% were G/G homozygous, 31.5% G/C heterozygous and 11.1% were C/C homozygous. The *SREBP-2* genotype frequencies deviated from the Hardy Weinberg Equilibrium ($X^2 = 6.39$, $p = 0.0114$). Mean values of TG ($p = 0.002$), TC ($p = 0.002$), ALT ($p = 0.04$) and AST ($p = 0.03$) levels were significantly higher in NAFLD subjects with G/C genotype as compared to G/G genotypes in obese and non-obese groups. Fasting insulin ($p = 0.03$), HOMA ($p = 0.009$) and hs-CRP levels were significantly higher in NAFLD subjects with G/C genotype as compared to obese and non obese subjects with G/G genotypes.

Conclusion: In this study, conducted for the first time in Asian Indians, *SREBP-2* 1784 G > C genotype was associated with NAFLD.

Keywords: Sterol regulatory element binding protein-2, Non-alcoholic fatty liver disease, Asian Indians, Alanine transaminase

1. Background

Non-alcoholic fatty liver disease (NAFLD) includes a spectrum of liver disorders characterized by accumu-

lation of hepatic fat in the absence of significant alcohol consumption. Further progression of NAFLD results in hepatic inflammation [nonalcoholic steatohepatitis (NASH)], which may result in cirrhosis [1]. Current evidence indicates that NAFLD is an important manifestation of insulin resistance [2]. In general, subjects with NAFLD generally are obese, have fasting hyperinsulinemia, and dysglycemia.

Estimates based on imaging and autopsy studies sug-

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gest that about 20% to 30% of adults in the United States and other Western countries have NAFLD [3]. According to our recent data, the prevalence of NAFLD is ~ 30% in Asian Indians in a selective sample from North India [4]. In a comparative study of Asian Indians vs. white Caucasians in USA, former had nearly 2.5 times hepatic triglyceride accumulation and significantly lower adiponectin levels [5]. Thus, there is preliminary evidence that fatty liver could be more severe in Asian Indians.

Sterol Response Element Binding Proteins (SREBPs) contain ~ 1,150 amino acids; SREBP-1 is responsible for negative regulation of the genes needed to make free fatty acid. *SREBP-2* is encoded by a separate gene on human chromosome 22q13 which spans 72 kb and is composed of 19 exons and 18 introns, and activates cholesterol biosynthesis in preference to fatty acid synthesis in liver and adipose tissues [6,7]. *SREBP* contributes to polygenic hypercholesterolemia [8]; in particular *SREBP-2* is an important candidate gene for hypercholesterolemia [9]. A common polymorphism detected using *MspI* is a G to C transversion at nucleotide position 1784 of the *SREBP-2* cDNA. *SREBP-2* 1784 G > C was significantly associated with high levels of serum total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) levels in Chinese population [10].

Genetic basis of NAFLD, and specifically *SREBP* gene, has been sparsely investigated. In this study we compared obese subjects with NAFLD, obese subjects without NAFLD and non obese subjects without NAFLD by analyzing clinical, anthropometric, metabolic profiles and *SREBP-2* gene polymorphism.

2. Methodology

2.1. Study subjects

This study was conducted in New Delhi (North India) from May 2007 to December 2010 after approval from the ethics committee. Subjects were recruited from the outpatient department of Fortis Hospital and department of Medicine, All India Institute of Medical Sciences, New Delhi. A total of 335 overweight subjects [Body mass index (BMI > 23 kg/m²)], 162 obese with NAFLD, 91 obese without NAFLD and 82 non-obese without NAFLD subjects were enrolled in this study. Recruitment of NAFLD was done on the basis of liver ultrasonography (see below). Age and sex matched obese and non-obese without NAFLD subjects

were recruited [11,12]. Subjects with known type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), presence of other liver diseases (alcoholic liver disease, viral hepatitis, autoimmune hepatitis, primary biliary cirrhosis, biliary obstruction, drug-induced liver damage etc.), severe end organ damage, human immunodeficiency virus infection, pregnancy and lactation were excluded from the study. A detailed history (demographic, social economic profiles, history of smoking, alcohol intake and physical activity patterns) and family history (T2DM, overweight, hypertension, liver disease and CVD) were obtained. A written informed consent was obtained.

2.2. Clinical and anthropometric measurements

Height, weight, waist circumference (WC), hip circumference (HC), waist-to hip ratio (W-HR) and skinfold thickness at 4 sites (triceps, biceps, suprailiac and subscapular) were measured according to standard protocols [11]. Body mass index (BMI) was calculated by dividing the weight (kg) by the height (m) squared and total skinfold (TSF) thickness was calculated as the sum of four skinfold thicknesses.

2.3. Biochemical analysis

Fasting blood samples were analyzed for blood glucose, lipid profile and liver function tests. Levels of fasting blood glucose (FBG), TC, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), alkaline phosphatase (ALK), aspartate transaminase (AST), alanine transaminase (ALT) and gamma-glutamyl transpeptidase (GGT) were assayed as previously described [4]. Fasting insulin levels were measured using commercially available radioimmunoassay insulin kits (Immunotech, France) [13]. High sensitivity C-reactive protein (hs-CRP) levels were measured by enzyme linked immunosorbent assay (ELISA) based kits (Biocheck Inc, CA, USA) [14]. The intra and interassay percentage coefficient variables were 1.3% and 2.1% for insulin and 2.3% and 3.1% for CRP, respectively.

2.4. Ultrasound imaging of liver

Liver ultrasound was carried out using 3.5 MHz curvilinear probe (Siemens-G 60 S 2004, Germany) by a trained operator who was blinded to all clinical and laboratory data. A complete examination requires both sub-costal and inter-costal scanning. The definition of fatty liver was based on a comparative assessment of image brightness relative to the kidneys, in line with previously reported diagnostic criteria [15].

2.5. Genetic analyses

Genomic DNA was extracted from peripheral blood leukocytes by rapid non-enzymatic method [16]. DNA amplification of the *SREBP-2* 1748 *G > C* polymorphism was performed using the following oligodeoxynucleotide primers (New England Biolab, USA); forward, GCCAGTGACCATTAACACCTTTTGA and reverse TCGTCTTCAAAGCCTGCCTCAGTGGCTGGC. PCR reaction was performed in a total volume of 25 μ l, containing 2.5 μ l of amplification buffer (100 mM Tris-HCl pH 9.0, 500 mM KCl, 15 mM MgCl₂ and 1.0% Triton X-100), 200 μ M each deoxy nucleotide (dATP, dCTP, dGTP, and dTTP), 10 pmol of each primer. PCR conditions for 1784 *G > C* were denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30s and extension at 72°C for 30s with a final extension at 72°C for 2 minutes.

The PCR (10 μ l) product was digested with 12 units of restriction enzyme *MspI* at 37°C for 6 hour in the buffer recommended by the manufacturer (New England Biolab, USA). The restriction fragments were electrophoresed in 20% polyacrylamide gels at 90 V for 4 h and directly visualized using silver staining.

3. Definitions

Overweight was defined as BMI \geq 23 kg/m² [11]. WC $>$ 90 cm for males and $>$ 80 cm for females was considered an indicator of abdominal obesity [11]. Impaired fasting glucose (IFG) was diagnosed according to American Diabetes Association criteria [12]. WC, males \geq 90 cm, females \geq 80 cm, FBG \geq 100 mg/dl, serum TG \geq 150 mg/dl, blood pressure \geq 130/85 mmHg and HDL-c; males \leq 40 mg/dl, and females \leq 50 mg/dl [12,17]. High level of hs-CRP was defined as $>$ 1 mg/L [14]. Insulin resistance was measured by two surrogate measures: fasting hyperinsulinemia and Homoeostasis model assessment (HOMA-IR). Hyperinsulinemia was defined as values in the highest quartile as described previously [18]. The value of HOMA denoting insulin resistance was termed as HOMA-IR and was calculated as = [fasting insulin (μ U/ml) \times fasting glucose (mmol/l)/22 \cdot 5]; [19]. AST value of up to 50 IU, ALT up to 50, ALK $>$ 80 IU and $<$ 240 IU were defined as normal.

4. Statistical analysis

Data were entered in an Excel spreadsheet (Microsoft Corp, Washington, USA). The distribution of biochem-

ical parameters were confirmed for approximate normality. We used mean and standard deviations and median (minimum- maximum) to summarize the variables. The differences in clinical and biochemical parameters in NAFLD, obese and non obese subjects were estimated by the ANOVA test.

The allelic and genotypic frequencies of *SREBP-2* gene of alleles and genotypes were determined by manual counting. In order to determine if observed allele frequency was in conformity with the expected frequency (Hardy Weinberg equilibrium), chi-square analysis was done. Between-group differences in proportions of alleles or genotypes were compared using Chi- square test and a two-tailed Fisher's exact test. Statistical analysis was done using STATA Version-9 (Stata Corp, USA) after confirming the normality aspect of quantitative variables, descriptive statistics were computed using Mean \pm SD and "t" test. Comparison of the genotypes of *SREBP-2* 1748 *G > C* and association with demographic and biochemical profiles of NAFLD, obese and non-obese subjects were compared using the student 't' test. Difference between proportions was tested using Chi- square test. P value $<$ 0.05 was considered as significant.

5. Results

A total of 335 subjects (238 males and 97 females) were recruited. Number of males among the NAFLD subjects was significantly higher as compared to obese and non-obese groups ($p <$ 0.05). Mean age of obese with NAFLD, obese without NAFLD and non-obese without NAFLD (38.2 ± 7.0 , 37.1 ± 6.9 and 36.1 ± 6.9 years, respectively) was comparable (Table 1).

5.1. Clinical profiles (Table 1)

Significantly higher values of systolic blood pressure ($p = 0.001$), diastolic blood pressure ($p = 0.003$), BMI ($p = 0.006$), WC ($p = 0.001$), HC ($p = 0.005$), and skinfolds thickness [triceps ($p = 0.03$), subscapular ($p = 0.001$), suprailiac ($p = 0.0001$) and total skinfolds ($p = 0.0001$)] were observed in NAFLD subjects as compared to obese and non-obese subjects.

5.2. Biochemical profiles (Table 1)

The levels of TG ($p = 0.002$), TC ($p = 0.002$), LDL-C ($p = 0.03$), VLDL ($p = 0.01$), ALT ($p = 0.0001$) and GGT ($p = 0.0001$) were significantly higher in NAFLD

Table 1
Demographic, clinical and biochemical profiles of obese with NAFLD, obese without NAFLD and non-obese without NAFLD subjects

Variables	Obese with NAFLD	Obese without NAFLD	Non-obese without NAFLD	p
Numbers (n)	162	91	82	
Age (yrs)	38.2 ± 7.0	37.1 ± 6.9	36.1 ± 6.9	0.08
Blood pressure (mmHg)				
Systolic	125.0 ± 11.8	119.3 ± 11.0	120.3 ± 11.0	0.001*
Diastolic	80.2 ± 8.6	77.4 ± 8.6	77.4 ± 8.6	0.003*
Body Mass Index (kg/m ²)	28.1 ± 3.2	26.8 ± 3.2	23.2 ± 3.2	0.006*
Waist circumference (cm)	94.5 ± 9.4	89.8 ± 9.1	85.8 ± 9.1	0.001*
Hip circumference (cm)	96.9 ± 7.2	94.5 ± 8.2	89.3 ± 8.2	0.005*
Waist hip ratio	0.93 ± 0.1	0.92 ± 0.1	0.90 ± 0.1	0.5
Skinfolds thickness (mm)				
Biceps	14.0 ± 5.1	13.0 ± 3.4	11.0 ± 3.4	0.4
Triceps	24.4 ± 8.3	19.8 ± 6.1	16.8 ± 6.1	0.03*
Subscapular	44.1 ± 8.6	39.4 ± 8.9	32.4 ± 8.9	0.001*
Suprailiac	46.8 ± 11.9	40.7 ± 10.3	33.7 ± 10.3	0.0001*
Total skin folds	129.3 ± 36.6	112.1 ± 28.9	92.1 ± 25.9	0.0001*
Blood glucose (mg/dl)				
Fasting	89.7 ± 12.0	87.2 ± 10.8	88.2 ± 10.8	0.2
Post-prandial	105.4 ± 10.9	104.7 ± 15.1	103.7 ± 12.1	0.3
Lipid Profiles (mg/dl)				
Triglyceride	172.0 ± 78.0	150 ± 65.3	148.0 ± 65.3	0.002*
Total cholesterol	189.1 ± 31.2	179.4 ± 26.8	177.4 ± 25.8	0.002*
HDL-C	39 ± 6.2	39.3 ± 10.8	36.3 ± 10.8	0.7
LDL-C	110.5 ± 22.9	104.9 ± 24.1	108.9 ± 24.1	0.03*
VLDL	33.4 ± 14.3	29.0 ± 14.0	26.0 ± 14.0	0.01*
Liver enzymes (I.U)				
ALT	38.7 ± 21.0	35.0 ± 9.7	32.0 ± 8.7	0.05*
AST	35.7 ± 19.2	33.6 ± 11.3	33.6 ± 11.3	0.2
ALK	136.0 ± 57.5	135.2 ± 61.6	134.2 ± 44.6	0.8
GGT	22.1 ± 11.6	18.1 ± 6.8	16.1 ± 5.8	0.0001*
Insulin (μU/ml)	11.7 ± 4.0	8.4 ± 3.3	7.3 ± 3.3	0.0008*
HOMA	2.5 ± 0.98	1.6 ± 0.8	1.4 ± 0.8	0.009*
Hs-CRP (μg/l)	3.2 ± 0.5	2.0 ± 0.4	1.96 ± 0.4	0.02*

Values are given as the mean ± standard deviation. P value < 0.05 is statistically significant; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; VLDL, very low-density lipoprotein; Alkaline phosphatase ALT, alanine transaminase; AST, aspartate transaminase; GGT, γ -glutamyl transpeptidase; HOMA-homoeostasis modal assessment for insulin resistance; hs-CRP, high sensitive C reactive protein.

subjects as compared to obese and non-obese subjects. Further, significantly high levels of fasting insulin ($p = 0.0008$), HOMA ($p = 0.009$) and hs-CRP ($p = 0.02$) were recorded in NAFLD subjects as compared to other groups (Table 1).

5.3. *SREBP-2*, 1784 G>C polymorphism (Table 2)

The overall frequency of G allele was 0.79 and of the C allele was 0.21. Frequency of the C allele was higher in the NAFLD subjects as compared to obese and non-obese subjects controls ($p = 0.01$). Frequency of C/C genotype was higher in NAFLD subjects as compared to obese and non-obese subjects ($p = 0.003$). In NAFLD subjects 57.4% were G/G homozygous, 31.5% G/C heterozygous and 11.1% were C/C ho-

mozygous. The *SREBP-2* genotype frequencies deviated from the Hardy Weinberg Equilibrium ($X^2 = 6.39$, $p = 0.0114$). The reproducibility of the genotyping data was checked by replicating the genotyping in a few randomly selected samples.

Comparison of the genotypes of *SREBP-2* 1784 G > C and association with clinical and biochemical profiles are presented in table 2. Mean values of TG ($p = 0.002$), TC ($p = 0.002$), ALT (0.04) and AST ($p = 0.03$) levels were significantly higher in NAFLD subjects with G/C genotype as compared to G/G genotypes in obese and non-obese groups. Fasting insulin ($p = 0.03$) and HOMA ($p = 0.009$) levels were significantly higher in NAFLD subjects with G/C genotype as compared to obese and non obese subjects with G/G genotype. Further, significantly higher levels of

Table 2

Comparison of the genotypes of *SREBP-2* $G > C$ and association with demographic and biochemical profiles of obese with NAFLD, obese without NAFLD and non-obese without NAFLD subjects

Variables	Obese with NAFLD		Obese without NAFLD		Non-obese without NAFLD		p
	G/G	G/C	G/G	G/C	G/G	G/C	
Numbers (%)	93 (57.41)	69 (42.59)	68 (75.14)	23 (24.86)	58 (70.7)	24(29.3)	
Age (yrs)	38 ± 6.2	38.3 ± 8.5	37.3 ± 6.9	39 ± 6.4	36.3 ± 6.9	35.1 ± 7.2	0.2
BMI (kg/m ²)	28.0 ± 2.7	28.6 ± 4.0	26.9 ± 3.2	26.7 ± 2.9	24.4 ± 3.2	23.7 ± 2.5	0.5
SBP (mmHg)	126.1 ± 11.1	124.5 ± 13.7	119.9 ± 11.4	119.8 ± 11.6	117 ± 8.9	118 ± 11.4	0.1
DBP (mmHg)	81.2 ± 6.7	79.8 ± 8.0	77.7 ± 8.7	76.4 ± 8.1	75.4 ± 8.1	77.7 ± 8.7	0.08
WC (cm)	94.3 ± 9.6	94.6 ± 9.7	90.0 ± 9.3	91.7 ± 10.9	89.9 ± 9.0	90.0 ± 9.3	0.5
HC (cm)	97 ± 7.7	97.3 ± 6.5	94.6 ± 7.8	94.4 ± 9.3	93.6 ± 7.0	94.4 ± 9.3	0.2
FBS (mg/dl)	89.3 ± 11.7	90 ± 11	88.2 ± 10.1	85.1 ± 10.2	83.8 ± 12.8	83.2 ± 10.1	0.4
PP (mg/dl)	106.0 ± 11.4	104.4 ± 11.6	105.1 ± 16.5	104.2 ± 12.9	103.3 ± 8.7	105.1 ± 16.5	0.1
TG (mg/dl)	160.7 ± 80.3	175.2 ± 78.2	142 ± 80.2	137 ± 60.2	114 ± 80.2	109 ± 53.2	0.002*
TC (mg/dl)	181.2 ± 32.6	194.6 ± 27.3	171.6 ± 28.3	174.7 ± 19.3	114 ± 80.2	109 ± 53.2	0.02*
LDL-C (mg/dl)	112.5 ± 24.4	110.7 ± 20.2	105.2 ± 25.6	104.2 ± 21.0	105.2 ± 25.6	105.2 ± 21.0	0.2
HDL-C (mg/dl)	39.5 ± 5.8	38.7 ± 9.1	39.6 ± 11.8	38.1 ± 6.8	37.6 ± 11.8	38.1 ± 6.8	0.4
VLDL (mg/dl)	32.5 ± 15	31 ± 11.2	26 ± 4.3	22.5 ± 9.9	21 ± 4.3	23 ± 4.4	0.5
ALT (IU/L)	37.7 ± 19.4	42.3 ± 18.8	35.9 ± 14.2	32.6 ± 13.3	32.6 ± 13.3	35.9 ± 14.2	0.04*
AST (IU/L)	36.9 ± 21.3	39.1 ± 26.5	34.7 ± 12.2	30.2 ± 7.4	30.2 ± 7.4	32.7 ± 12.2	0.03*
ALK (IU/L)	135.5 ± 58.0	137.8 ± 61.4	134.4 ± 63.3	131.3 ± 65.1	123.4 ± 63.3	121.8 ± 51.1	0.4
GGT (IU/L)	20 ± 4.5	22.5 ± 6.8	18.2 ± 6.5	18 ± 3.6	18.2 ± 6.5	19 ± 3.6	0.5
Insulin (μU/ml)	7.7 ± 0.6	10.4 ± 2.5	6.3 ± 2.3	7.6 ± 0.3	5.3 ± 2.3	6.6 ± 0.3	0.04*
HOMA	2.3 ± 0.8	3.1 ± 0.9	1.6 ± 0.3	1.6 ± 0.3	1.5 ± 0.3	1.64 ± 0.3	0.03*
CRP (μg/l)	2.7 ± 0.6	5.2 ± 0.7	2.4 ± 0.6	3.3 ± 1.8	2.5 ± 0.6	2.0 ± 2.1	0.009*

Values are given as the mean ± standard deviation. P value < 0.05 is statistically significant, BMI-body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference; HC, hip circumference; FBS, fasting blood glucose; PP, post prandial blood glucose; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; VLDL, very-low density lipoprotein; ALK, Alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; GGT, γ -glutamyl transpeptidase; HOMA-homoeostasis modal assessment for insulin resistance; hs-CRP, high sensitive C reactive protein.

hs-CRP were recorded in NAFLD subjects with G/C genotype as compared to G/G genotype in both the groups ($p = 0.009$).

6. Discussion

This is the first report to assess the *SREBP-2* (1784 G/C) polymorphism in subjects with NAFLD in Asian Indians. We observed a significantly higher frequency of the C allele of *SREBP-2* 1784 $G > C$ in the obese NAFLD subjects. Our findings are similar to that observed among hypercholesterolemic subjects in other Asian populations, especially in Switzerland [8] and France [20].

Asian Indians have higher percentage body fat, abdominal obesity, and insulin resistance than white Caucasians [21,22]. It has been opined that that all factors associated with NAFLD such as generalized obesity, abdominal obesity, T2DM, hypertriglyceridaemia and insulin resistance are highly prevalent in urban Asian Indians and may be important for the pathogenesis of NAFLD in this ethnic group.

Willner et al. [23] found that 18% of patients with NASH had an affected first degree relative. However, clustering could simply be a reflection of the well established heritability of the risk factors for NAFLD, obesity and dyslipidemia. Browning and Caldwell reported that the ethnic differences in the prevalence of NAFLD and NAFLD-related 'cryptogenic' cirrhosis strongly suggest a genetic susceptibility to NAFLD rather than to its risk factors [24,25]. Further, the ethnic susceptibility also shown by threefold higher prevalence of cryptogenic cirrhosis in Hispanic and fourfold lower African-Americans as compared with European-American patients despite a similar prevalence of T2DM [24].

The serum TC levels of the individuals carrying the G/C genotype were higher than those of the G/G genotype in NAFLD group. The 1784 G/C polymorphism was also reported to have a significant impact on TC level in the hypercholesterolemic subjects from Switzerland and Israel [8]. However, this effect was not found in normocholesterolemic individuals. On the other hand, Robinet et al. [20] have demonstrated that there was only a trend between TC values and the 1784 $G > C$ polymorphism in a selected population of

men with a cardiovascular risk from France when hypercholesterolemic and normocholesterolemic subjects were considered separately.

Horton et al. [26] reported that over-expression of *SREBP* tends to favor the synthesis of cholesterol over triglyceride in liver of transgenic mice. Previous reports suggest that an increase in the amount of *SREBP-2* in the liver is associated with an overproduction of cholesterol [27]. Usually fatty liver associates with worse glucose and plasma triglyceride. Interestingly, serum levels of TG and ALT were significantly higher in NAFLD subjects with *G/C* genotype in the present study.

SREBP-2 involvement in NASH and hyperinsulinemia is a common component of the pathophysiology of NASH that stems from insulin resistance. In this present study we showed that fasting insulin and HOMA-IR levels were significantly associated with *G/C* genotype in NAFLD subjects. These findings provide a potential explanation for the close relationship between insulin resistance and *SREBP-2* gene polymorphism.

CRP is a strong marker for atherosclerotic disease and cardiovascular events [28,29], and its levels are elevated in the metabolic syndrome and with visceral adiposity [30]. The findings of our study of elevated CRP in NAFLD subjects with *G/C* genotype suggest a possible association of *SREBP-2* polymorphism and CRP in NAFLD and needs to be assessed further.

A limitation of this study is the lack of data on siblings and other ancestral members of the recruited subjects which could help in determining the effect of population stratification. Clinically it is desirable to identify a marker linked with increased risk of NAFLD. Further, the diagnosis of NAFLD was based on liver ultrasound, which may not be as accurate as liver biopsy in identifying NAFLD.

7. Conclusion

The C allele and the *G/C* genotype of *SREBP-2* 1784 *G > C* is associated with increased risk of NAFLD in Asian Indians residing in north India and should be evaluated further in future prospective studies.

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Conflict of interest

Authors declare no conflict of interest.

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