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Original Article

Association of polymorphisms of *adiponectin* gene promoter-11377C/G, *glutathione peroxidase-1* gene C594T, and cigarette smoking in nonalcoholic fatty liver disease

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Abstract

Background: The number of studies on *adiponectin*, *GPx-1* gene polymorphisms, and nonalcoholic fatty liver disease (NAFLD) susceptibility is increasing, but none have investigated the effect of cigarette smoking in combination with the gene polymorphisms on the susceptibility to NAFLD. In order to understand the distribution of adiponectin and GPx-1 in the local population, to explore the possible association of cigarette smoking with *adiponectin* and *GPx-1* gene polymorphisms in the pathogenesis of NAFLD, we conducted this research, examining the distribution of polymorphisms of *adiponectin* and *GPx-1* in NAFLD patients and healthy controls, analyzing the association between these polymorphisms and cigarette smoking.

Methods: Two hundred nonalcoholic simple fatty liver (NAFL), 200 nonalcoholic steatohepatitis (NASH), and 200 nonalcoholic fatty hepatic cirrhosis (NAFHC) cases from the First Affiliated Hospital of Xinxiang Medical College in China from February 2011 to November 2014 were selected for this study, and 200 healthy individuals as a control group. No significant difference among the four groups in age, sex, ethnicity, and birthplace was observed. The genetic polymorphisms of *adiponectin* gene promoter-11377C/G and *GPx-1* gene C594T were analyzed using polymerase chain reaction-restriction fragment length polymorphisms in peripheral blood leukocytes of the above-mentioned cases. The interaction between the two mutants and the gene-environment association of the genotypes with cigarette smoking were analyzed.

Results: The frequencies of *adiponectin* gene promoter-11377C/G(CG), -11377C/G (GG), *GPx-1* gene C594T (CT) and C594T (TT) were 24.50%, 26.00%, 24.00%, and 25.50% in the NAFL group, 34.50%, 37.00%, 35.00%, and 36.00% in the NASH group, 42.00%, 46.00%, 43.50%, and 45.50% in the NAFHC group, and 14.00%, 14.50%, 13.00%, and 14.00% in the control group, respectively. Statistical tests showed a significant difference in the frequencies among each group ($p < 0.01$). The risk of NAFLD significantly increased in patients with *adiponectin* gene promoter-11377C/G (CG) genotype [odds ratio $(OR)_{NAFL} = 2.5278$; $OR_{NASH} = 6.1823$; $OR_{NAFHC} = 17.8570$), in those with -11377C/G (GG) genotype ($OR_{NAFL} = 2.5900$; $OR_{NASH} = 6.4017$; $OR_{NAFHC} = 18.9023$), in those with *GPx-1* gene *C594T* (CT) genotype ($OR_{NAFL} = 2.6687$; $OR_{NASH} = 6.7772$; $OR_{NAFHC} = 22.2063$), and in those with C594T (TT) genotype ($OR_{NAFL} = 2.6330$; $OR_{NASH} = 6.4729$; $OR_{NAFHC} = 21.5682$). Combined analysis of the polymorphisms showed that percentages of *adiponectin* gene promoter -11377C/G (GG)/*GPx-1* gene C594T (TT) in the NAFL, the NASH, NAFHC, and control groups was 7.00%, 13.50%, 21.00%, and 2.00%, respectively ($p < 0.01$). The people who carried the *adiponectin* gene promoter -11377C/G (GG)/*GPx-1* gene *C594T* (TT) had a high risk of NAFLD ($OR_{NAFL} = 7.2800$; $OR_{NASH} = 41.2941$; $OR_{NAFHC} = 363.9724$), and statistical analysis suggested a positive association between -11377C/G (GG) and C594T (TT) in increasing the risk of NAFLD ($\gamma_{2NAFL} = 2.2071$, $\gamma_{4\ NAFL} = 2.0773$; $\gamma_{2\ NASH} = 2.1084$; $\gamma_{4NASH} = 2.0543$; $\gamma_{2\ NAFHC} = 2.1387$; $\gamma_{4NAFHC} = 2.0004$). Likewise, there were also positive association in the pathogenesis of NAFLD between -11377C/G (CG) and C594T (TT), -11377C/G (CG) and C594T (CT), -11377C/G (GG), and C594T (TT) (CT).The frequencies of smoking index (SI) ≤ 400 and SI > 400 were 22.50% and 26.50% in the NAFL group, 29.00% and 40.50% in the NASH group, 34.00% and 51.50% in the NAFHC group, and 15.50% and

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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12.00% in the control group, respectively. Statistical tests showed a significant difference in the frequencies among each group (all $p < 0.01$). The risk of NAFLD significantly increased in patients with SI $\leq$ 400 ($OR_{NAFL} = 2.0636$; $OR_{NASH} = 4.4474$; $OR_{NAFH\ C} = 10.9677$) and in those with SI > 400 ($OR_{NAFL} = 3.1393$; $OR_{NASH} = 8.0225$; $OR_{NAFHC} = 21.4583$), and statistical analysis suggested a positive association between cigarette smoking and -11377C/G (CG), -11377C/G (GG), *C594T* (CT), and *C594T* (TT) in increasing the risk of NAFLD (all $\gamma > 1$).
Conclusion: *Adiponectin* gene promoter -11377C/G (CG), -11377C/G (GG), *GPx-1* gene C594T (CT), C594T (TT), and cigarette smoking are risk factors in NAFLD, and the significant association between genetic polymorphisms of -11377C/G, C594T, and cigarette smoking amplify the risk of NAFLD.

Keywords: adiponectin gene promoter -11377C/G; cigarette smoking; glutathione peroxidase-1 gene C594T; nonalcoholic fatty liver disease; polymorphism

1. Introduction

The exact mechanism of nonalcoholic fatty liver disease (NAFLD) is unclear, but the two-hit theory is more popular than other hypotheses. The theory states that insulin resistance can cause liver fat accumulation in hepatocytes, the first hit in the pathogenesis of NAFLD, which makes the liver more vulnerable to oxidative stress and subsequent lipid peroxidation, among the factors constituting the second hit, thus progressing to inflammation and fibrosis, and eventually NAFLD.[1,2]

Cigarettes release nicotine and carbon monoxide in the combustion process. These ingredients interfere with lipid metabolism, and multiple ingredients of cigarettes also stimulate and promote free radical lipid peroxidation, which participates in the development of NAFLD.[3] Adiponectin, one of the major adipocyte-secreted proteins, has attracted scientific interest in recent years and has been extensively studied in both human and animal models. Adiponectin exerts insulin-sensitizing effects through binding to adiponectin receptors, leading to activation of adenosine monophosphate-activated protein kinase, peroxisome proliferators activated receptor-α, and potentially other unknown molecular pathways. The role of adiponectin in improving insulin sensitivity is such that it has become an important factor in inhibiting the progress of NAFLD.[4] Glutathione peroxidase-1 (GPx-1) has the function of scavenging free radicals and derivatives, and phospholipid hydroperoxide glutathione peroxidase constitutes with catalase and glutathione-S-transferase (an organic hydroperoxide reduction system) at different levels of substrate specificity, reducing the formation of lipid peroxides and enhancing resistance to oxidation damage, an important factor that controls the progression of NAFLD.[5] *Adiponectin* and *GPx-1* genes have polymorphisms which have multiple alleles with different alleles encoding different adiponectin or GPx-1 activities. Polymorphisms in *adiponectin* or *GPx-1* genes can affect the reaction of the body to the external environment (such as smoking), which is an important factor that determines its susceptibility to NAFLD.

The number of studies on *adiponectin*, *GPx-1* gene polymorphisms, and NAFLD susceptibility is increasing, but none have investigated the effect of cigarette smoking in combination with the gene polymorphisms on the susceptibility to NAFLD. In order to understand the distribution of adiponectin and GPx-1 in the local population, to explore the possible association of cigarette smoking with *adiponectin* and *GPx-1* gene polymorphisms in the pathogenesis of NAFLD, we conducted this research examining the distribution of polymorphisms of *adiponectin* and *GPx-1* in NAFLD patients and healthy controls, and analyzed the relationship between these polymorphisms and smoking status.

2. Methods

2.1. Diagnostic criteria

The diagnosis of NAFLD was based on guidelines for diagnosis and treatment of NAFLD revised by the Fatty Liver and Alcoholic Liver Disease Study Group of the Chinese Liver Disease Association in 2010.[6]

2.2. Inclusion criteria

(1) The patients conformed to the above diagnostic criteria of NAFLD; (2) the patients were aged from 17 years to 69 years, men or women; (3) alanine transaminase or aspartate transaminase level was less than 80 U/L; (4) the patients voluntarily signed an informed consent form and passed ethic evaluation.

2.3. Exclusion criteria

(1) History of alcohol intake > 20g/d; (2) co-existence of other liver diseases, such as viral, drug-induced, auto-immune hepatitis, and so on; (3) suspicion of liver cirrhosis or liver cancer; (4) co-existence of other severe systematic disease or infectious disease, such as malignant neoplasm, severe cardiopulmonary disease, neurological disorders, human immunodeficiency virus infection, and so on; (5) currently pregnant, breastfeeding, pregnancy anticipated during study, or planning to conceive; (6) co-existence of mental disorders or severe neurosis, or unable to express symptoms subjectively, and hindering of connection and cooperation with researchers because of dysgnosia or aphasis.

2.4. Case and control selection

Based on the above-mentioned diagnostic, inclusion, and exclusion criteria, 200 nonalcoholic simple fatty liver (NAFL), 200 nonalcoholic steatohepatitis (NASH), and 200 nonalcoholic fatty hepatic cirrhosis (NAFHC) cases were selected for this study from the Department of Gastroenterology of the First Affiliated Hospital of Xinxiang Medical University from February 2011 to November 2014. The control group consisted of 200 healthy people, excluding persons with type 2 diabetes, obesity, hypertension, hyperlipidemia, and other metabolic disorders, as well as those with a history of drinking. There was no significant difference between the four groups in age, sex, ethnicity, and birthplace. The NAFLD cases and healthy controls were all divided into nonsmokers and smokers, the latter identified based on the criteria of taking at least one cigarette per day for more than 6 months. Smoking status was estimated by smoking index (SI) = daily cigarette consumption (piece) × duration of cigarette smoking (year). According to SI value, the smokers were divided into SI $\leq$ 400 and SI > 400 subgroups.

2.5. Sample collection

Blood samples (2–3 mL) were collected into ethylenediaminetetraacetic acid tubes containing an anticoagulant. After centrifugation, three blood fractions were obtained: plasma, red blood cells, and a buffy layer containing white blood cells. DNA was extracted from the white blood cells using the QIAamp DNA extraction kit (Qiagen, Hilden, Germany) and stored at −30°C for the following experiments.

2.6. Analysis of polymorphisms of adiponectin gene promoter-11377C/G

The analysis of -11377C/G polymorphism was conducted by reference to the method of Gupta et al.[7] The primer sequences were: 5′-TGGTGGACTTGACTTTACTG-3′ (upstream primer) and 5′-CAGCCTG GAGAACTGGAA-3′ (downstream primer), both synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. The polymerase chain reaction (PCR) reaction system included: 1 μL of genomic DNA, 0.5 μL of upstream and downstream primers, 25 μL of Taq DNA polymerase Mix (Dalian Takara Biotechnology Co., Ltd, Liaoning Province, China). The reaction conditions were: predegeneration at 94°C for 5 minutes; 94°C degeneration for 40 seconds, and annealing at 55°C 45 seconds, 72°C for 30 seconds, 40 cycles; the last extension at 72°C for 7 minutes. The length of PCR products was 329 bp. Bio-Rad staining of agarose gel electrophoresis confirmed the amplification results. The PCR products were digested with restriction endonuclease Hha I. The enzyme digestion products were observed with ultraviolet gel imager, in which three genotypes were visible: homozygous CC (329 bp band), homozygous GG (211 and 118 bp bands), and heterozygote CG (329 bp, 211 bp, and 118 bp bands Fig. 1).

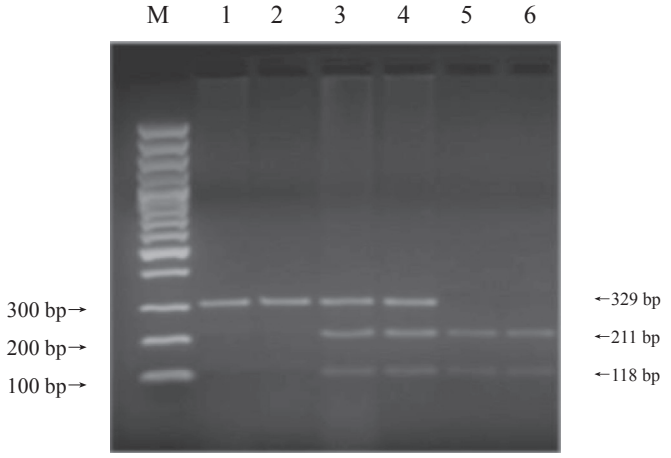

Fig. 1. The electrophoretogram of PCR products indicating the -11377 variants of adiponectin gene promoter.

2.7. Analysis of GPx-1 gene C594T polymorphism

The analysis of *GPx-1* gene C594T polymorphism was conducted by reference to the method of Suzen et al.[8] The primer sequences were: 5′-CCTACGCAGGTACAGCC-3′ (upstream primer), 5′-CAACAGGACCAGCACCCATCTC-3′ (downstream primer), synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. Application of TaKaRa PCR amplification kit (Dalian Takara Biotechnology Co.) PCR was conducted in 25 μL reactions, containing 1-μL template DNA, 2.5 μL10 × PCR buffer, 2 L deoxynucleotide mixture, 0.125 μL TaKaRa Taq DNA polymerase, 15pmol primers, and 17.375-μL sterilized deionized water. The PCR reaction conditions were as follows: predegeneration at 93°C for 3 minutes; 93°C degeneration for 1 minute, annealing at 66°C for 1 minute, at 70°C for 1 minute, 35 cycles; and extension at 70°C for 10 minutes. The length of PCR product was 240 bp. PCR reaction products (12 μL) were incubated with restriction endonuclease Dde at 37°C in a water bath. Enzyme-digested products were analyzed with 3.5% agarose gel electrophoresis (containing 0.5 μg/mL ethidium bromide). The electrophoretogram showed homozygous C594T (CC) (240 bp band), homozygous C594T (TT) (163 bp and 77 bp bands), and heterozygous C594T (CT) (240 bp, 163 bp, and 77 bp bands; Fig. 2).

2.8. Statistical analysis

SPSS statistical package for Windows 11.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Groups were tested to determine whether they were in Hardy–Weinberg equilibrium, with $p > 0.05$ indicating compliance with Hardy–Weinberg law. The odds ratio (OR) and 95% confidence interval were used to evaluate the relative risk between the NAFLD group and the control group, with the gene frequency and allele frequency analyzed using χ^2 test. A p value < 0.05 was considered statistically significant.

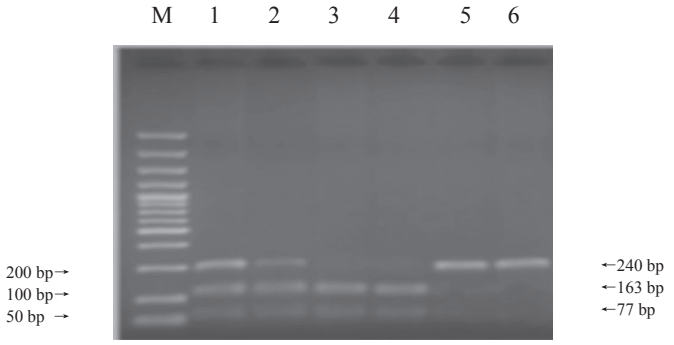

Fig. 2. The electrophoretogram of PCR products indicating the *glutathione peroxidase*-1 gene C594T polymorphism.

Conditional logistic regression was applied to analyze interaction, with the following criteria according to the model of interaction and interaction coefficients of Khoury and Wagener ($\gamma = \beta_{eg}/\beta_e$) in determining gene–environment interaction.[9] Criteria 1 were: $\gamma > 1$ indicates that the effect of environmental exposure was amplified by genetic factors, i.e., positive interaction; $\gamma < 1$ indicates that the effect of environmental exposure was reduced by genetic factors, i.e., negative interaction; $\gamma = 1$ indicates that gene and environmental exposure had no interaction. In a case-control study, γ equaled to $\lg OR_{eg}$ divided by $\lg OR_e$. Criteria 2 were: $OR_{eg} = OR_e \times OR_g$ is a multiplicative model; $OR_{eg} > OR_e \times OR_g$ is a super-multiplicative model; $OR_{eg} < OR_e \times OR_g$ for the multiplicative model; $OR_{eg} = OR_e + OR_g - 1$ is an additive model.

3. Results

3.1. General information of NAFLD group and control group

There was no significant difference among each group in age and sex. The body mass index, waist circumference, hip circumference, waist-to-hip ratio, systolic blood pressure, diastolic blood pressure, fasting blood glucose, aspartate transaminase, alanine transaminase, triglyceride, serum total cholesterol, low density lipoprotein cholesterol, fasting serum insulin, and high sensitivity C-reactive protein in the NAFL, NASH, and NAFHC groups were all significantly higher than that in the control group ($p < 0.01$), while high-density lipoprotein cholesterol was significantly lower than that in the control group ($p < 0.01$; Table 1).

3.2. Related analysis of NAFLD susceptibility and smoking

The frequencies of SI $\leq$ 400 and SI > 400 were 22.50% and 26.50% in NAFL cases, 29.00% and 40.50% in NASH cases, 34.00% and 51.50% in NAFHC cases, and 15.50% and 12.00% in healthy controls, respectively. Statistical tests showed significant difference in the frequencies among each group (all $p < 0.01$). The risk of NAFLD significantly increased in subjects with SI $\leq$ 400 ($OR_{NAFL} = 2.0636$; $OR_{NASH} = 4.4474$; $OR_{NAFHC} = 10.9677$) and in those with SI > 400 ($OR_{NAFL} = 3.1393$; $OR_{NASH} = 8.0225$; $OR_{NAFHC} = 21.4583$). The NAFLD incidence of SI > 400 was significantly higher than that of SI $\leq$ 400 ($p < 0.01$; Table 2).

Table 1
General information of the nonalcoholic fatty liver disease and control groups [n (%)].

Items	Control group	NAFLD group					
		NAFL group	*p*	NASH group	*p*	NAFHC group	*p*
Sex, *n* (%)			0.1905		0.2147		0.1932
Male	120 (60.00)	121 (60.50)		119 (59.50)		117 (58.50)	
Female	80 (40.00)	79 (39.50)		81 (40.50)		83 (41.50)	
Age (y)	54.71 ± 4.89	54.72 ± 5.73	0.2318	54.63 ± 9.25	0.2842	54.91 ± 4.53	0.1726
BMI (kg/m^2)	48.47 ± 14.57	55.87 ± 4.29	0.0094	65.51 ± 11.35	0.0082	76.39 ± 10.71	0.0073
WC (cm)	73.06 ± 7.05	84.46 ± 9.57	0.0071	95.52 ± 9.79	0.0063	103.84 ± 12.81	0.0057
HC (cm)	76.91 ± 15.11	84.75 ± 11.23	0.0091	98.40 ± 13.61	0.0076	112.52 ± 17.75	0.0052
WHR	0.77 ± 0.09	0.87 ± 0.04	0.0082	0.93 ± 0.03	0.0075	1.05 ± 0.12	0.0065
SBP (mmHg)	123.89 ± 15.81	137.35 ± 16.39	0.0072	148.46 ± 22.37	0.0058	158.16 ± 12.81	0.0049
DBP (mmHg)	74.16 ± 10.37	85.57 ± 16.08	0.0096	95.67 ± 26.14	0.0085	1125.38 ± 26.52	0.0079
FBG (mmol/L)	5.54 ± 1.29	6.42 ± 1.54	0.0077	7.82 ± 1.97	0.0056	7.82 ± 1.97	0.0046
AST (U/L)	24.36 ± 11.28	35.63 ± 13.71	0.0083	47.63 ± 15.86	0.0062	59.39 ± 18.24	0.0051
ALT (U/L)	18.50 ± 9.63	30.51 ± 1214	0.0085	37.91 ± 17.07	0.0068	53.91 ± 13.62	0.0048
TG (mmol/L)	1.25 ± 0.47	1.97 ± 1.01	0.0095	2.58 ± 1.38	0.0075	3.82 ± 1.56	0.0042
TC (mmol/L)	4.29 ± 1.87	5.34 ± 1.62	0.0087	6.64 ± 2.61	0.0072	7.51 ± 3.68	0.0059
LDL-C (mmol/L)	2.40 ± 1.51	2.97 ± 1.39	0.0084	3.27 ± 1.14	0.0061	3.27 ± 1.14	0.0032
HDL-C (mmol/L)	1.93 ± 0.84	1.52 ± 0.47	0.0063	1.35 ± 0.56	0.0047	1.03 ± 0.27	0.031
FINS (mIU/L)	6.38 ± 2.18	8.65 ± 3.94	0.0072	12.65 ± 3.17	0.0059	15.47 ± 6.98	0.0025
hs-CRP (mg/L)	2.36 ± 0.52	2.94 ± 1.05	0.0081	3.85 ± 1.21	0.0047	3.85 ± 1.21	0.0039

ALT = alanine transaminase; AST = aspartate transaminase; BMI = body mass index; DBP = diastolic blood pressure; FBG = fasting blood glucose; FINS = fasting serum insulin; HC = hip circumference; HDL-C = high-density lipoprotein cholesterol; hs-CRP = high sensitivity C-reactive protein; LDL-C = low density lipoprotein cholesterol; NAFL = nonalcoholic simple fatty liver; NAFHC = non-alcoholic fatty hepatic cirrhosis; NAFLD = nonalcoholic fatty liver disease; NASH = nonalcoholic steatohepatitis; SBP = systolic blood pressure; TC = serum total cholesterol; TG = triglyceride; WC = waist circumference; WHR = waist-to-hip ratio..

Table 2
Related analysis of nonalcoholic fatty liver disease susceptibility and smoking.

Groups	Smoking status, *n* (%)		
	Nonsmoking	SI ≤ 400	SI > 400
Control group (*n* = 200)	145 (72.50)	31 (15.50)	24 (12.00)
NAFL group (*n* = 200)	102 (51.00)	45(22.50)	53 (26.50)
OR[a] $_{NAFL}$	1.00	2.0636	3.1393
95% CI $_{NAFL}$		1.2317–5.2186	1.9354–5.2175
p $_{NAFL}$		0.0092	0.0089
NASH group (*n* = 200)	61 (30.50)	58 (29.00)	81 (40.50)
OR[a] $_{NASH}$	1.00	4.4474*	8.0225*
95% CI $_{NASH}$		2.5081–7.2194	4.4763–11.2165
p $_{NASH}$		0.0085	0.0072
NAFHC group (*n* = 200)	29 (14.50)	68 (34.00)	103 (51.50)
OR[a] $_{NAFHC}$	1.00	10.96771*,**	21.4583*,**
95% CI $_{NAFHC}$		6.2647–14.3210	16.2627–32.3215
p_{NAFHC}		0.0063	0.0047

* Compared with OR[a] $_{NAFL}$, $p < 0.01$.
** Compared with OR[a] $_{NASH}$, $p < 0.01$.
CI = confidence interval; NAFHC = nonalcoholic fatty hepatic cirrhosis; NAFL = nonalcoholic simple fatty liver; NASG = nonalcoholic steatohepatitis; OR = odds ratio; SI = smoking index.
[a] Adjusted according to sex, age, body mass index, waist circumference, hip circumference, waist-to-hip ratio, systolic blood pressure, diastolic blood pressure, fasting blood glucose, aspartate transaminase, alanine transaminase, triglyceride, total cholesterol, low density lipoprotein cholesterol, fasting serum insulin, and high sensitivity C-reactive protein.

3.3. Distribution of polymorphisms of adiponectin gene promoter-11377C/G and GPx-1 gene C594T polymorphism

The genotype distribution of -11377C/G in the control group complied with the Hardy–Weinberg law ($p > 0.05$), indicating representativeness of this group (Table 3). There was a significant difference among each group in CC, CG, and GG genotype frequency ($p < 0.01$), and in the distribution of G allele ($p < 0.01$), the OR value was greater than 1, the risk of NAFLD with the allele G was significantly higher than the allele C. C594T genotype, and allele frequency is in accordance with the above rules (Table 4).

3.4. Interaction of the two polymorphisms in NAFLD

The percentages of -11377C/G (GG) C594T (TT) in NAFL, NASH, NAFHC, and control groups were 7.00%, 13.50%, 21.00%, and 2.00%, respectively ($p < 0.01$). The people who carried with -11377C/G (GG)/C594T (TT) had a high risk of NAFLD ($OR_{NAFL} = 7.2800$; $OR_{NASH} = 41.2941$; $OR_{NAFHC} = 363.9724$), and statistical analysis suggested a positive interaction between -11377C/G (GG) and C594T (TT) in increasing the risk of NAFLD($\gamma_2 = \beta_{2*4}/\beta_4$, $\gamma_4 = \beta_{2*4}/\beta_2$; $\gamma_{2NAFL} = 2.2071$, $\gamma_{4NAFL} = 2.0773$; $\gamma_{2\ NASH} = 2.1084$, $\gamma_{4NASH} = 2.0543$; $\gamma_{2\ NAFHC} = 2.1387$,$\gamma_{4NAFHC} = 2.0004$). Likewise, there were also positive associations in the pathogenesis of NAFLD between -11377C/G (CG) and C594T (TT), -11377C/G (CG) and C594T (CT), -11377C/G (GG), and C594T (CT) (Table 5).

3.5. Interaction of the two polymorphisms with cigarette smoking in NAFLD

The OR value by simple SI ≤ 400 exposure (OR_{e1}) was 2.2189 in the NAFL group, 4.7273 in the NASH group, and 12.6061 in the NAFHC group, the OR value by simple -11377C/G (CG) exposure (OR_{g1}) was 2.5267 in the NAFL group, 6.1176 in the NASH group, and 19.8095 in the NAFHC group, when SI ≤ 400 and -11377C/G (CG) existed simultaneously, the interaction OR_{e1g1} was 5.8367 in the NAFL group, 30.5882 in the NASH group, and 251.3332 in the

Table 3
Distribution of polymorphisms of adiponectin gene promoter-11377C/G genotypes and allele.

Groups	Genotype, *n* (%)			Allele, *n* (%)	
	CC	CG	GG	C	G
Control (*n* = 200)	143 (71.50)	28 (14.00)	29 (14.50)	314 (78.50)	86 (21.50)
NAFL (*n* = 200)	99 (49.50)	49 (24.50)	52 (26.00)	247 (61.75)	153 (38.25)
OR[a] $_{NAFL}$	1.00	2.5278	2.5900	1.00	2.2617
95% CI $_{NAFL}$		1.6347–4.2191	1.7380–4.7036		1.2041–3.8664
p $_{NAFL}$		0.0089	0.0085		0.0097
NASH (*n* = 200)	57 (28.50)	69 (34.50)	74 (37.00)	183 (45.75)	217 (54.25)
OR[a] $_{NASH}$	1.00	6.1823*	6.4017*	1.00	4.3295*
95% CI $_{NASH}$		3.1762–9.3258	3.4726–9.8042		2.3790–7.9782
p $_{NASH}$		0.0079	0.0072		0.0086
NAFHC(*n* = 200)	24 (12.00)	84 (42.00)	92 (46.00)	132 (33.00)	268 (67.00)
OR[a] $_{NAFHC}$	1.00	17.8750*,**	18.9023*,**	1.00	7.4130*,**
95% CI $_{NAFHC}$		9.3769–25.4301	11.3714–26.30852		4.3017–10.1854
p $_{NAFHC}$		0.0052	0.0049		0.0074

* Compared with OR[a] $_{NAFL}$, $p < 0.01$.
** Compared with OR[a] $_{NASH}$, $p < 0.01$.
CI = confidence interval; NAFHC = nonalcoholic fatty hepatic cirrhosis; NAFL = nonalcoholic simple fatty liver; NASG = nonalcoholic steatohepatitis; OR = odds ratio; SI = smoking index.
[a] Adjusted according to sex, age, body mass index, waist circumference, hip circumference, waist-to-hip ratio, systolic blood pressure, diastolic blood pressure, fasting blood glucose, aspartate transaminase, alanine transaminase, triglyceride, total cholesterol, low density lipoprotein cholesterol, fasting serum insulin, and high sensitivity C-reactive protein, and smoking status.

Table 4
Distribution of GPx-1 gene C594T polymorphism genotypes and allele.

Groups	Genotype, *n* (%)			Allele, *n* (%)	
	CC	CT	TT	C	T
Control ($n = 200$)	146 (73.00)	26 (13.00)	28 (14.00)	318 (79.50)	82 (20.50)
NAFL ($n = 200$)	101 (50.50)	48 (24.00)	51 (25.50)	250 (62.50)	150 (37.50)
OR^{a}_{NAFL}	1.00	2.6687	2.6330	1.00	2.3268
95% CI_{NAFL}		1.5342–4.6813	1.5107–4.7136		1.3153–3.8719
p_{NAFL}		0.0087	0.0091		0.0094
NASH ($n = 200$)	5 8(29.00)	70 (35.00)	72 (36.00)	186 (46.50)	214 (53.50)
OR^{a}_{NASH}	1.00	6.7772*	6.4729*	1.00	4.4618*
95% CI_{NASH}		3.4196–10.2115	3.1795–10.2181		2.3782–8.1506
p_{NASH}		0.0071	0.0075		0.0081
NAFHC ($n = 200$)	22 (11.00)	87 (43.50)	91(45.50)	131 (32.75)	269 (67.25)
OR^{a}_{NAFHC}	1.00	22.2063***	21.5682***	1.00	7.9633***
95% CI_{NAFHC}		12.2647–29.3264	12.2657–31.1237		4.3706–9.1724
p_{NAFHC}		0.0047	0.0045		0.0063

* Compared with OR^{a}_{NAFL}, $p < 0.01$.
** Compared with OR^{a}_{NASH}, $p < 0.01$.
CI = confidence interval; NAFHC = nonalcoholic fatty hepatic cirrhosis; NAFL = nonalcoholic simple fatty liver; NASG = nonalcoholic steatohepatitis; OR = odds ratio; SI = smoking index.
[a] Adjusted according to sex, age, body mass index, waist circumference, hip circumference, waist-to-hip ratio, systolic blood pressure, diastolic blood pressure, fasting blood glucose, aspartate transaminase, alanine transaminase, triglyceride, total cholesterol, low density lipoprotein cholesterol, fasting serum insulin, and high sensitivity C-reactive protein, and smoking status.

NAFHC group, the interaction coefficient $\gamma = \beta_{e1g1}/\beta_{e1} > 1$, $OR_{e1g1} > OR_{e1} \times OR_{g1}$, showing a super-multiplicative model, and statistical analysis also suggested positive interactions between SI $\leq$ 400 and -11377C/G (GG), SI > 400 and -11377C/G (CG), and SI > 400 and -11377C/G (GG) in increasing the risk of NAFLD ($\gamma > 1$; Table 6). Similarly, there were also positive associations between SI $\leq$ 400 and C594T (CT), SI $\leq$ 400 and C594T (TT), SI > 400 and C594T (CT), and SI > 400 and C594T (TT) in increasing the risk of NAFLD ($\gamma > 1$; Table 7).

4. Discussion

Adiponectin has been identified as one of the most abundant adipose-specific adipokines and plays a critical role in the maintenance of insulin sensitivity and inhibiting liver fat deposition.[10] The expression of adiponectin is controlled by genes and induced by environmental factors, and its inducible expression levels exhibit individual differences. The human *adiponectin* gene is located on chromosome 3q27, containing three exons and two introns, encoding 244 amino acids. There are a variety of single-nucleotide polymorphisms in the *adiponectin* gene, such as -11377C/G, +276G/T, and +45T/G. The -11377C/G polymorphism is considered the main meaningful polymorphism in the *adiponectin* gene, affecting the activity of the promoter and decreasing the expression of adiponectin in blood and tissues, thereby affecting its biological effect. The *adiponectin* gene -11377C/G polymorphism has three genotypes, namely -11377C/G (CC), -11377C/G (CG), and -11377C/G (GG). Studies have suggested a correlation between the -11377C/G polymorphism and metabolic syndrome.[11,12] This study found that the risk of NAFLD significantly increased in patients with the -11377C/G (CG) genotype (OR_{NAFL} = 2.5278; OR_{NASH} = 6.1823; OR_{NAFHC} = 17.8570), and in those with the -11377C/G (GG) genotype (OR_{NAFL} = 2.5900; OR_{NASH} = 6.4017; OR_{NAFHC} = 18.9023). The mechanism of the -11377C/G (GG) genotype being susceptible to NAFLD is not clear; a related study showed that the G allele may decrease the expression of adiponectin through inactivating *adiponectin* gene transcription, thus decreasing insulin sensitivity,[13] and finally causing lipid deposition in the liver, raising the risk of NAFLD.

GPx, an enzyme dependent on the micronutrient selenium, plays a critical role in the reduction of lipid and hydrogen peroxide. There are four subspecies of GPx that catalyze the reduction of hydrogen peroxide in specific tissue locations. GPx-1 is ubiquitous and found in the cytosol of most cells, including red blood cells. GPx-2 is also cytosolic but is confined to the gastrointestinal tract. GPx-3 occurs in plasma as a glycoprotein, and GPx-4 interacts with complex lipids, such as cholesterol and lipoproteins damaged by free radicals, and is found in mitochondria.[14] Human *GPx-1* gene is located on chromosome 3p21.3, containing two exons and one intron. *GPx-1* gene C594T polymorphism (rsl050450), which resides in the coding region and results in an amino acid substitution of proline with leucine (Pro198Leu), was reported to be associated with a reduction of GPx-1 activity.[15] Studies have shown that *GPx-1* gene C594T polymorphism may increase the incidence of oxidative stress-related diseases such as prostate cancer and bladder cancer.[16,17] This study found that the frequencies of C594T (CT) and C594T (TT) genotype in the NAFL, NASH, and NAFHC groups were significantly higher than that in control group (all $p < 0.01$), and the risk of NAFLD significantly increased in patients with C594T (CT) genotype (OR_{NAFL} = 2.6687; OR_{NASH} = 6.7772;

Table 5
Interaction of polymorphisms of adiponectin gene promoter -11377C/G and GPx-1 gene C594T in nonalcoholic fatty liver disease [n (%)].

Groups	Combined genotypes of -11377C/G and C594T, *n* (%)]								
	CC/CC	CC/CT	CC/TT	CG/CC	CG/CT	CG/TT	GG/CC	GG/CT	GG/TT
Control ($n = 200$)	104 (52.00)	19 (9.50)	20 (10.00)	20 (10.00)	4 (2.00)	4 (2.00)	22 (11.00)	3 (1.50)	4 (2.00)
NAFL ($n = 200$)	50 (25.00)	24 (12.00)	25 (12.50)	25 (12.50)	12 (6.00)	12 (6.00)	26 (13.00)	12 (6.00)	14 (7.00)
OR^{a}_{NAFL}	1.00	2.6274[b](OR_1)	2.6000[c](OR_2)	2.6000[d](OR_3)	6.2400[e](OR_{1*3})	6.2400[f](OR_{2*3})	2.4582[g](OR_4)	8.3200[h](OR_{1*4})	7.2800[i](OR_{2*4})
95% CI_{NAFL}		1.3372−4.2108	1.2374−5.2183	1.2374−5.2183	3.9017−9.8561	3.9017−9.8561	1.0374−5.2516	5.0376−11.2487	4.0349−11.2124
β_{NAFL}		0.4195(β_1)	0.4150(β_2)	0.4150(β_3)	0.7959(β_{1*3})	0.7959(β_{2*3})	0.3906(β_4)	0.9201(β_{1*4})	0.8621(β_{2*4})
NASH ($n = 200$)	17 (8.50)	20 (10.00)	20 (10.00)	20 (10.00)	24 (12.00)	25 (12.50)	21 (10.50)	26 (13.00)	27 (13.50)
OR^{a}_{NASH}	1.00	6.4396[b](OR_1)*	6.1176[c](OR_2)*	6.1176[d](OR_3)*	36.7059[e](OR_{1*3})*	38.2343[f](OR_{2*3})*	5.8396[g](OR_4)*	53.0191[h](OR_{1*4})*	41.2941i(OR_{2*4})*
95% CI_{NASH}		3.4274−10.2195	3.3418−9.9047	3.0725−9.2172	25.7906−43.9718	26.0307−45.3295	2.6086−9.7419	32.6529−76.8971	28.0369−65.2114
β_{NASH}		0.8089(β_1)*	0.7866(β_2)*	0.7866(β_3)*	1.5647(β_{1*3})*	1.5824(β_{2*3})*	0.7664(β_4)*	1.7244β_{1*4})*	1.6159(β_{2*4})*
NAFHC($n = 200$)	3 (1.50)	10 (5.00)	11 (5.50)	9 (4.50)	37 (18.50)	38 (19.00)	10 (5.00)	40 (20.00)	42 (21.00)
OR^{a}_{NAFHC}	1.00	18.2456[b](OR_1)*,**	19.0667[c](OR_2)*,**	15.6000[d](OR_3)*,**	320.6667[e](OR_{1*3})*,**	329.3333[f](OR_{2*3})*,**	15.7576[g](OR_4)*,**	462.2219[h](OR_{1*4})*,**	363.9724[i](OR_{2*4})*,**
95% CI_{NAFHC}		11.2610−27.5473	11.7805−31.2596	9.0328−23.8758	270.3032−417.1824	282.1474−425.2187	9.0392−25.2471	398.5173−563.2182	297.0388−465.4694
β_{NAFHC}		1.2611(β_1)*,**	1.2803(β_2)*,**	1.1931(β_3)*,**	2.5061(β_{1*3})*,**	2.5176(β_{2*3})*,**	1.1975(β_4)*,**	2.6649(β_{1*4})*,**	2.5611(β_{2*4})*,**

* Compared with OR^{a}_{NAFL}, $p < 0.01$.

** Compared with OR^{a}_{NASH}, $p < 0.01$.

CI = confidence interval; NAFHC = nonalcoholic fatty hepatic cirrhosis; NAFL = nonalcoholic simple fatty liver; NASG = nonalcoholic steatohepatitis; OR = odds ratio.

[a] Adjusted according to sex, age, body mass index, waist circumference, hip circumference, waist-to-hip ratio, systolic blood pressure, diastolic blood pressure, fasting blood glucose, aspartate transaminase, alanine transaminase, triglyceride, total cholesterol, low density lipoprotein cholesterol, fasting serum insulin, and high sensitivity C-reactive protein, and smoking status.

[b] OR value by simple GPx-1-C594T heterozygote exposure (OR_1).

[c] OR value by simple GPx-1-C594T homozygous mutant exposure (OR_2).

[d] OR value by simple -11377C/G heterozygote exposure (OR_3).

[e] OR value by the interaction of GPx-1-C594T heterozygote and -11377C/G heterozygote (OR_{1*3}).

[f] OR value by the interaction of GPx-1-C594T homozygous mutant and -11377C/G heterozygote (OR_{2*3}).

[g] OR value by simple -11377C/G homozygous mutant exposure (OR_4).

[h] OR value by the interaction of GPx-1-C594T heterozygote and -11377C/G homozygous mutant (OR_{1*4}).

[i] OR value by the interaction of GPx-1-C594T homozygous mutants and -11377C/G homozygous mutants (OR_{2*4}).

Table 6
Interaction of -11377C/G polymorphism of adiponectin gene promoter and cigarette smoking in nonalcoholic fatty liver disease [n (%)].

Groups	−11377C/G genotype and smoking status, *n* (%)								
	CC+ nonsmoking	CC+ SI ≤ 400	CC+ SI > 400	CG+ nonsmoking	CG+ SI ≤ 400	CG+ SI > 4 00	GG+ nonsmoking	GG+ SI ≤ 400	GG+ SI > 400
Control ($n = 200$)	104 (52.00)	22 (11.00)	17 (8.50)	21 (10.50)	4 (2.00)	3 (1.50)	20 (10.00)	5 (2.50)	4 (2.00)
NAFL ($n = 200$)	49 (24.50)	23 (11.50)	27 (13.50)	25 (12.50)	11 (5.50)	13 (6.50)	28 (14.00)	11 (5.50)	13 (6.50)
OR[a] $_{NAFL}$	1.00	2.2189[b](OR_{e1})	3.3710[c](OR_{e2})	2.5267[d](OR_{g1})	5.8367[e](OR_{e1*g1})	9.1973[f](OR_{e2*g1})	2.9714[g](OR_{g2})	4.6694[h](OR_{e1*g2})	6.8980[i](OR_{e2*g2})
95% CI_{NAFL}		1.1347−4.8873	2.1075−5.8338	1.1374−5.1646	3.1385−9.2760	6.6540−11.2760	1.2974−5.9502	2.5975−9.0752	3.5076−10.5825
β_{NAFL}		0.3461(β_{e1})	0.5278(β_{e2})	0.4026(β_{g1})	0.7662(OR_{e1*g1})	0.9637(β_{e2*g1})	0.4730(β_{g2})	0.6693(β_{e1*g2})	0.8387(β_{e2*g2})
NASH ($n = 200$)	17(8.50)	17 (8.50)	23 (11.50)	21 (10.50)	20 (10.00)	28 (14.00)	23 (11.50)	21 (10.50)	30 (15.00)
OR[a] $_{NASH}$	1.00	4.7273[b](OR_{e1})*	8.2768[c](OR_{e2})*	6.1176[d](OR_{g1})*	30.5882[e](OR_{e1*g1})*	57.0980[f](OR_{e2*g1})*	7.0353g(ORg2)*	25.6941[h](OR_{e1*g2})*	45.8824[i](OR_{e2*g2})*
95% CI_{NASH}		2.1984−7.5307	4.8470−11.6043	4.5095−9.0598	19.3982−43.7804	42.8375−75.8043	3.6937−10.4085	16.0491−37.0938	34.8027−65.5538
β_{NASH}		0.6746(β_{e1})*	0.9179(β_{e2})*	0.7866(β_{g1})*	1.4856(OR_{e1*g1})*	1.7566(β_{e2*g1})*	0.8473(β_{g2})*	1.4098(β_{e1*g2})*	1.6616(β_{e2*g2})*
NAFHC ($n = 200$)	3 (1.50)	8 (4.00)	13 (6.50)	12 (6.00)	29 (14.50)	43 (2150)	14 (7.00)	31 (15.50)	47 (23.50)
OR[a] $_{NAFHC}$	1.00	12.6061[b](OR_{e1})*,**	26.5098[c](OR_{e2})*,**	19.8095[d](OR_{g1})*,**	251.3332[e](OR_{e1*g1})*,**	496.8889[f](OR_{e2*g1})*,**	24.2667[g](OR_{g2})*,**	214.9333[h](OR_{e1*g2})*,**	407.3332[i](OR_{e2*g2})*,**
95% CI $_{NAFHC}$		8.0724−19.5573	17.2628−32.4241	12.4194−25.9582	197.4072−375.9306	368.8164−579.0725	16.7806−35.2714	156.7430−2895.0426	362.6923−475.3762
β_{NAFHC}		1.1006(β_{e1})*,**	1.42324(β_{e2})*,**	1.2969(β_{g1})*,**	2.4002(OR_{e1*g1})*,**	2.6963(β_{e2*g1})*,**	1.3850(β_{g2})*,**	2.3323(β_{e1*g2})*,**	2.6099(β_{e2*g2})*,**

* Compared with OR[a] $_{NAFL}$, $p < 0.01$.

** Compared with OR[a] $_{NASH}$, $p < 0.01$.

CI = confidence interval; NAFHC = nonalcoholic fatty hepatic cirrhosis; NAFL = nonalcoholic simple fatty liver; NASG = nonalcoholic steatohepatitis; OR = odds ratio; SI = smoking index.

[a] Adjusted according to sex, age, body mass index, waist circumference, hip circumference, waist-to-hip ratio, systolic blood pressure, diastolic blood pressure, fasting blood glucose, aspartate transaminase, alanine transaminase, triglyceride, total cholesterol, low density lipoprotein cholesterol, fasting serum insulin, and high sensitivity C-reactive protein.

[b] OR value by simple SI ≤ 400 exposure (OR_{e1}).

[c] OR value by simple SI > 400 exposure (OR_{e2}).

[d] OR value by simple heterozygote type exposure (OR_{g1}).

[e] OR value by the interaction of SI ≤ 400 and heterozygote (OR_{e1g1}).

[f] OR value by the interaction of SI > 400 and heterozygote (OR_{e2g1}).

[g] OR value by simple homozygous mutant type exposure (OR_{g2}).

[h] OR value by the interaction of SI ≤ 400 and homozygous mutant (OR_{e1g2}).

[i] OR value by the interaction of SI > 400 and homozygous mutant (OR_{e2g2}).

Table 7
Interaction of GPx-1 gene C594T polymorphism and cigarette smoking in nonalcoholic fatty liver disease [n (%)].

Groups	C594T genotype and smoking status, *n* (%)								
	CC+ nonsmoking	CC+ SI $\leq$ 400	CC+ SI > 400	CT+ nonsmoking	CT+ SI $\leq$ 400	CT+ SI > 400	TT+ non-smoking	TT+ SI $\leq$ 400	TT+ SI > 400
Control (n = 200)	106 (53.00)	23 (11.50)	17 (8.50)	19 (9.50)	4 (2.00)	3 (1.50)	20 (10.00)	4 (2.00)	4 (2.00)
NAFL (n = 200)	51 (25.50)	23 (11.50)	27 (13.50)	24 (12.00)	11 (5.50)	13 (6.50)	27 (13.50)	11 (5.50)	13 (6.50)
OR[a] $_{NAFL}$	1.00	2.0784[b](OR_{e1})	3.3010[c](OR_{e2})	2.6254[d](OR_{g1})	5.7157[e](OR_{e1*g1})	9.0065[f](OR_{e2*g1})	2.8059[g](OR_{g2})	5.7157[h](OR_{e1*g2})	6.7549[i](OR_{e2*g2})
95% CI $_{NAFL}$		1.2165−3.4716	1.8975−5.4706	1.8794−5.4036	3.2059−8.8945	6.9137−12.5496	1.4649−5.2871	3.7930−8.5063	3.9592−9.5945
β_{NAFL}		0.3177(β_{e1})	0.5186(β_{e2})	0.4192(β_{g1})	0.7571(OR_{e1*g1})	0.9546(β_{e2*g1})	0.4481(β_{g2})	0.7571(β_{e1*g2})	0.8296(β_{e2*g2})
NASH (n = 200)	18 (9.00)	17 (8.50)	23 (11.50)	21 (10.50)	20 (10.00)	29 (14.50)	22 (11.00)	21 (10.50)	29 (14.50)
OR[a] $_{NASH}$	1.00	4.3527[b](OR_{e1})*	7.9673[c](OR_{e2})*	6.5088[d](OR_{g1})*	29.4444[e](OR_{e1*g1})*	56.9260[f](OR_{e2*g1})*	6.4778[g](OR_{g2})*	30.9167[h](OR_{e1*g2})*	42.6944[i](ORe2*g2)*
95% CI $_{NASH}$		2.5725−7.1792	4.7594−11.9703	3.2926−10.4238	17.5902−41.9794	41.6439−75.5394	3.2375−9.2084	21.6724−45.2871	29.6598−55.2037
β_{NASH}		0.6388(β_{e1})*	0.9013(β_{e2})*	0.8135(β_{g1})*	1.4690(OR_{e1*g1})*	1.7553(β_{e2*g1})*	0.8114(β_{g2})*	1.4902(β_{e1*g2})*	1.6304(β_{e2*g2})*
NAFHC (n = 200)	3 (1.50)	7 (3.50)	12 (6.00)	12 (6.00)	30 (15.00)	45 (22.50)	14 (7.00)	31 (15.50)	46 (23.00)
OR[a] $_{NAFHC}$	1.00	10.7536[b](OR_{e1})*·**	24.9412[c](OR_{e2})*·**	22.3158[d](OR_{g1})*·**	265.0000[e](OR_{e1*g1})*·**	529.7583[f](OR_{e2*g1})*·**	24.7333[g](OR_{g2})*·**	273.8279[h](OR_{e1*g2})*·**	406.3297[i](OR_{e2*g2})*·**
95% CI $_{NAFHC}$		6.5913−17.8692	17.6328−35.8407	16.7291−33.4827	198.6917−305.9704	432.6493−652.8715	15.6108−32.5838	194.3328−365.2179	327.3965−513.0576
β_{NAFHC}		1.0316(β_{e1})*·**	1.3969(β_{e2})*·**	1.3486(β_{g1})*·**	2.4232(OR_{e1*g1})*·**	2.7241(β_{e2*g1})*·**)	1.3933(β_{g2})*·**	2.4375(β_{e1*g2})*·**	2.6089(β_{e2*g2})*·**

* Compared with OR[a] $_{NAFL}$, $p < 0.01$.

** Compared with OR[a] $_{NASH}$, $p < 0.01$.

CI = confidence interval; NAFHC = nonalcoholic fatty hepatic cirrhosis; NAFL = nonalcoholic simple fatty liver; NASG = nonalcoholic steatohepatitis; OR = odds ratio; SI = smoking index.

[b] OR value by simple SI $\leq$ 400 exposure (OR_{e1}).

[c] OR value by simple SI > 400 exposure (OR_{e2}).

[d] OR value by simple heterozygote type exposure (OR_{g1}).

[e] OR value by the interaction of SI $\leq$ 400 and heterozygote (OR_{e1g1}).

[f] OR value by the interaction of SI > 400 and heterozygote (OR_{e2g1}).

[g] OR value by simple homozygous mutant type exposure (OR_{g2}).

[h] OR value by the interaction of SI $\leq$ 400 and homozygous mutant (OR_{e1g2}).

[i] OR value by the interaction of SI > 400 and homozygous mutant (OR_{e2g2}).

[a] Adjusted according to sex, age, body mass index, waist circumference, hip circumference, waist-to-hip ratio, systolic blood pressure, diastolic blood pressure, fasting blood glucose, aspartate transaminase, alanine transaminase, triglyceride, total cholesterol, low density lipoprotein cholesterol, fasting serum insulin, and high sensitivity C-reactive protein.

$OR_{NAFHC} = 22.2063$) and in those with C594T (TT) genotype ($OR_{NAFL} = 2.6330$; $OR_{NASH} = 6.4729$; $OR_{NAFHC} = 21.5682$), which corresponded with the results of other related researches.

This study found that the interaction of -11377C/G and *GPx-1* gene C594T mutation increased the risk for NAFLD. The -11377C/G (GG) and C594T (TT) genotypes in NAFLD had super-multiplicative effect ($\gamma_{2NAFL} = 2.2071$, $\gamma_{4NAFL} = 2.0773$; $\gamma_{2NASH} = 2.1084$, $\gamma_{4NASH} = 2.0543$; $\gamma_{2NAFHC} = 2.1387$, $\gamma_{4NAFHC} = 2.0004$). Likewise, there were also positive interactions in the pathogenesis of NAFLD between -11377C/G (CG) and C594T (TT), -11377C/G (CG) and C594T (CT), and -11377C/G (GG) and C594T (CT). The risk of NAFLD significantly increased in individuals with SI $\leq$ 400 ($OR_{NAFL} = 2.0636$; $OR_{NASH} = 4.4474$; $OR_{NAFHC} = 10.9677$) and in those with SI > 400 ($OR_{NAFL} = 3.1393$; $OR_{NASH} = 8.0225$; $OR_{NAFHC} = 21.4583$), and statistical analysis suggested positive interactions between cigarette smoking and -11377C/G (CG), -11377C/G (GG), C594T(CT), and C594T (TT) in increasing the risk of NAFLD (all $\gamma > 1$). The OR_{eg} of each of the two genotypes and smoking exposure was greater than $OR_e \times OR_g$, indicating that the two homozygous mutant genes and smoking interact in a supermultiplicative manner in the pathogenesis of NAFLD. Long-term smoking can cause oxidation of glucose metabolism in cells, significantly weaken nonoxidative pathways, and increase free fatty acids levels in plasma, and which can be taken up by liver and adipose tissue and synthesize triglycerides, leading to the development of insulin resistance. The nicotine in tobacco can cause sympathetic excitement, leading to an increased release of catecholamines and glucagon, which are potent antagonists of insulin action. The impaired insulin action creates the conditions for the first hit in the pathogenesis of NAFLD.[18,19] Usually, the body can clean out excess free radicals thanks to the existence of the free radical scavenging system including GPx-1, while a variety of compounds in cigarettes can increase the levels of harmful free radicals. In the bodies of long-term smokers, excessive oxidizing free radicals travel through the blood into the liver cells, inducing lipid peroxidation and free radical reaction, and eventually leading to fatty liver. This may be the reason why smoking can promote the risk of NAFLD by -11377C/G (CG), -11377C/G (GG), C594T (CT), and C594T (TT).

NAFLD is a complex process involving interaction of environmental factors and multiple genes. This study suggests that carriers of adiponectin gene promoter-11377C/G (CG), -11377C/G (GG), and *GPx-1* gene C594T (CT) and C594T (TT) genotypes may have a high risk of NAFLD, and the gene genotypes can interact with cigarette smoking in the pathogenesis of NAFLD. In order to achieve effective prevention of NAFLD, measures controlling environmental factors, such as smoking cessation, need to be taken.

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