Association Between Tfeb Gene Polymorphism, Gene–environment Interaction, and Fatty Liver Disease: a Case–control Study in China

Chunbao Mo (molipol@163.com)
Guilin Medical University https://orcid.org/0000-0003-0738-7773
Tingyu Mai
Guilin Medical University
Jiansheng Cai
Guangxi Medical University
Haoyu He
Guangxi Medical University
Huaxiang Lu
Guangxi Medical University
Xu Tang
Guangxi Medical University
Quanhui Chen
Guangxi Medical University
Xia Xu
Guangxi Medical University
Chuntao Nong
Guangxi Medical University
Shuzhen Liu
Guangxi Medical University
Dechan Tan
Guilin Medical University
Qiumei Liu
Guangxi Medical University
Min Xu
Guangxi Medical University
You Li
Guilin Medical University
Zhiyong Zhang
Guilin Medical University
Jian Qin
Guangxi Medical University

Research

Keywords: TFEB, gene polymorphism, gene–environment interaction, fatty liver disease

Posted Date: May 25th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-541251/v1

Abstract

Background: Fatty liver disease (FLD) is a serious public health problem that is rapidly increasing. Evidences indicated that the transcription factor EB (*TFEB*) gene may be involved in the pathophysiology of FLD; however, whether *TEFB* polymorphism is association with FLD remains unclear.

Objectives: To explore the association among *TFEB* polymorphism, gene–environment interaction, and FLD and provide epidemiological evidence for clarifying the genetic factors of FLD.

Methods: This study is a case–control study. Sequenom MassARRAY was applied in genotyping. Logical regression was used to analyze the association between *TFEB* polymorphism and FLD, and the gene–environment interaction in FLD was evaluated by multiplication and additive interaction models.

Results: (1) The alleles and genotypes of each single nucleotide polymorphism of *TFEB* in the case and control groups were evenly distributed; no statistically substantial difference was observed. (2) Logistic regression analysis indicated that *TFEB* polymorphism is not remarkably associated with FLD. (3) In the multiplicative interaction model, rs1015149, rs1062966, and rs11754668 had remarkable interaction with smoking amount. Rs1062966 and rs11754668 also had a considerable interaction with body mass index and alcohol intake, respectively. However, no remarkable additive interaction was observed.

Conclusion: *TFEB* polymorphism is not directly associated with FLD susceptibility, but the risk can be changed through gene–environment interaction.

Introduction

Health problem has gradually aroused people's concern with the advancement of economy and society and the improvement of living standard. Fatty liver disease (FLD) is a chronic disease and a serious public health problem that is rapidly increasing.[1, 2] FLD is the pathological process of excess adipose accumulation in liver cells, caused by many factors, such as disease and drug. Simple hepatic steatosis may transform into steatohepatitis or cirrhosis as FLD progresses. According to etiology, FLD is classified as nonalcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD). Studies have indicated that the pathogenesis of FLD is affected by abnormal fat metabolism, immune response, environment, genetic, and other factors.[3] Currently, specific medicine for FLD is deficient; the effective prevention and control measures for FLD are early detection and intervention, including diet control and exercise; and alcohol abstinence is the chief measure for patients with ALD.[3]

Similar with most diseases, FLD is influenced by environmental and genetic factors. Single nucleotide polymorphisms (SNPs) are the most common form of mutations in the human genome. Studies have found that SNPs are associated with FLD. Wen et al. suggested the association between rs780094 polymorphism and NAFLD in Uyghur population by case–control method.[4] Luigi et al. also reported that rs738409 polymorphism in pNPLA3 may be a genetic variant that is associated with NAFLD and ALD. [5]

In recent years, transcription factor EB (TFEB) has attracted extensive attention in the study of autophagy mechanism. *TFEB* is the main gene involved in lysosome biosynthesis and encodes TFEB, which is an important regulatory factor for autophagy and lysosomal biosynthesis. TFEB is considered the main activator for autophagy–lysosomal gene transcription and refers to inflammation,[6] cell autophagy,[7] lipid metabolism[8] and other biological processes. Previous researches indicated that TFEB can regulate the expression of many genes related to lipid degradation, such as cluster of differentiation 36, fatty acid binding proteins, and carnitine acetyltransferase. Furthermore, TFEB can regulate lipid degradation factor, peroxisome proliferator-activated receptor alpha (PPARα), and an upstream factor of PPARα, proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α), through signal-mediated transfer to the nucleus; thus, PPARα is affected to participate in lipid metabolism.[9, 10]

Increasing TFEB levels in vivo may protect mice liver from alcohol-induced damage,[11] and promoting TFEB-mediated lysosomal biogenesis using formononetin can ameliorate the fatty disease process in mice liver.[12] Although increasing studies have provided etiological evidence to elucidate the mechanism between TFEB and fatty liver, few researches have focused on the relationship between *TFEB* and FLD. Therefore, in this study, a case–control approach was adopted to explore the association between *TFEB* polymorphism and FLD, and gene–environment interaction was evaluated to provide epidemiological evidence of the genetic factors of FLD.

Materials And Methods

2.1. Study design and population

This case–control study included 228 patients with FLD diagnosed by ultrasonography according to the diagnostic guidelines released by the Chinese Medical Association. Individuals with liver diseases, tumors, and autoimmune diseases caused by drugs and viruses were excluded. A total of 342 healthy individuals who were matched by sex and age (with variation of ±3 years) in a proportion of 1:1.5 were selected as the control group. All the participants were permanent residents in Gongcheng County, Guilin City, Guangxi Zhuang Autonomous Region, People's Republic of China and signed the informed consent voluntarily after fully understanding the research content and importance of this project. Our research protocol was approved by the Ethics Committee of Guilin Medical University.

2.2 Data collection

All the participants were required to answer a questionnaire from a trained researcher to collect information on demography, behavior, exercise, disease history, nutritional diet, and other data. Behavioral factors include smoking and alcohol consumption. The amount of smoking is expressed in pack year, that is, the number of packs (20 cigarettes per bag) per day multiplied by the number of years of smoking. In addition to daily alcohol intake, we also assessed the daily intake of 11 types of food, including cereals and their products, potatoes, vegetables, and fruits, via dietary survey. The participants were also examined by professional physicians to collect anthropometric indicators, such as height, weight, waist circumference, and blood pressure. Venous blood was collected for subsequent tests and experiments.

2.3 Biochemical testing

Two venous blood samples were collected from each participant. One set of samples was tested for biochemical indicators, including triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT), and uric acid (UA), at a local hospital. The other set of samples was used in the SNP typing experiment.

2.4 Selection and genotyping of SNPs

Functional and validated SNP screening strategies were utilized to screen the target SNPs. This strategy focused on important functional loci and supplemented by susceptible loci. Finally, the rs1015149, rs1062966, rs14063, rs2273068, and rs11754668 of the *TFEB* gene were selected as the target SNPs for genotyping.

Genomic DNA was isolated from venous blood using a commercial DNA extraction kit (Tiangen, Beijing, China). SNP genotyping was performed using the Sequenom MassARRAY matrix-assisted laser desorption ionization time-of-flight mass spectrometry platform (Sequenom, Inc., San Diego, CA, USA). The primers were designed and synthesized by Bio Miao Biological Technology Co., Ltd. (Table S1).

2.5 Statistical analysis

Descriptive statistics for continuous and categorical variables were conducted using mean ± standard deviation (SD) and frequency (proportion), respectively. Student's t-test and chi-square test were applied to compare the differences among two

groups and genotype subgroups. Pearson's chi-square test was utilized to evaluate the Hardy–Weinberg equilibrium (HWE) before analyzing SNP data. The samples were considered representative when $p > 0.05$. We performed logistic regression to estimate the effects of genotypes and gene–environment multiplicative interactions on FLD, and odds ratio (OR) and 95% confidence interval (95% CI) were calculated. The test level $\alpha = 0.05$. However, logistic regression was limited to estimate additive interactions; hence, relative excess risk due to interaction (RERI), attributable proportion of interaction (AP), synergy index (SI), and their 95% CIs were calculated. Additive interactions were considered statistically significant when the 95% CI of RERI and AP did not include 0 and the 95% CI of SI did not contain 1.[13] SPSS 25.0 (IBM, Chicago, IL, USA) and PLINK 1.90 software were used to implement general statistical analysis and gene polymorphism analysis. In addition, R software 4.0.2 and "epiR" package were utilized to complete the calculation of RERI, AP, and SI.

Results

3.1. Characteristics of the participants

The demographic and behavioral characteristics of the participants are listed in Table 1. The subjects have a total number of 570 and a roughly equal gender proportion. The age range is 30–83 years with an average of 58.15 years. The majority of the subjects (78.42%) belong to Yao population. No remarkable differences in gender, age, ethnicity, marital status, hypertension, smoking, drinking, and other factors were observed between the control and case groups at baseline ($p > 0.05$). However, the proportion of subjects with a history of hypertension and the average daily sitting time were significantly higher in the case group than in the control group ($p < 0.05$).

The dietary situation is shown in Table 2. Vegetables and cereals and their products were the main daily dietary intake of the participants. No significant difference was observed in the daily food intake of the two groups ($p > 0.05$).

The clinical indicators are exhibited in Table 3. The two groups showed no statistically significant difference in AST ($t = -1.415$, $p = 0.158$). However, HDL-C was significantly lower in the case group than in the control group, and the other indicators were significantly higher in the case group compared with the control group ($p < 0.05$).

Results indicate that the demographic, behavioral, and dietary variables in the two groups were matched preferably.

3.2 Basic information of SNPs

The rs1015149, rs1062966, rs11754668, rs14063, and rs2273068 of *TFEB* are located in chromosome 6, and the minimum allele frequency of each locus was greater than 0.05. The success rate of genotyping was very high at nearly 100% by MassARRAY. All the SNPs' loci were consistent with HWE ($p_{HWE} > 0.05$); therefore, the study subjects are representative (Table S2).

3.3 Genotypic frequency

The alleles and genotypes of each SNP of *TFEB* in the two groups were evenly distributed. No statistically significant difference was observed ($p > 0.05$, Table 4).

3.4 Associations between genotypes and FLD

FLD was regarded as the dependent variable. Co-dominant, dominant, and recessive models were used for logistic regression analysis using gender and age as adjustment factors. The result indicated that no significant correlations exist between genotypes and FLD in the co-dominant, dominant, and recessive models ($p > 0.05$, Table 5).

3.5 Interactions between environmental factors and SNP in FLD

Multiplicative and additive models were used to evaluate the interactions of each SNP locus with environmental factors, including diabetes, smoking amount, alcohol intake, daily sitting time, waist circumference, and body mass index (BMI). Compared with single genes, some gene–environment interactions were remarkably associated with FLD susceptibility. In the multiplicative interaction model, rs1015149, rs1062966, and rs11754668 had substantial interaction with smoking amount. Among them, rs1062966 and rs11754668 also had remarkable interaction with BMI and alcohol intake, respectively.

Notably, the 95% CIs of RERI and AP contained 0, and the 95% CI of SI contained 1; thus, all gene–environment additive interactions with FLD were not statistically significant (Table 6, Table S3).

Discussion

In this case–control study, we analyzed the association of *TFEB* polymorphisms and FLD and assessed gene–environment interactions to provide epidemiological evidence of the genetic factors related to the occurrence and development of FLD. Results showed that the alleles and genotypes of each SNP of *TFEB* in the case and control groups were evenly distributed; no statistically significant difference was observed. Logistic regression analysis indicated that *TFEB* polymorphism is not substantially associated with FLD. Previous studies have suggested that autophagy plays an important role in maintaining liver homeostasis.[14] *TFEB* knockout in mice may result in the hepatic accumulation of fatty acid-β and impaired oxidation in hepatocytes, which lead to elevated fatty acid and glycerol levels and lipid metabolism disorders in hepatocytes.[15] According to the results, *TFEB* may be involved in the pathophysiological basis of FLD. However, the relationship between *TFEB* polymorphism and FLD was not observed in this study; thus, these SNPs may not affect the normal expression of *TFEB*.

Gene–environment interaction plays an important role in the occurrence and development of complex diseases, such as FLD. Zhu et al. demonstrated that the gene–gene interaction between *AGTR1* and *PPARγ* is associated with the occurrence of NAFLD in Chinese population.[16] Zhang et al. found that people with 11391G/A(AA) and EC-SOD (CG+GG) genotypes suffer a higher risk of NAFLD, and these genotypes have an interaction with *Helicobacter pylori* infection.[17] Therefore, the analysis of gene–environment interaction might be conducive to understand etiological factors and guide the prevention and treatment of FLD. The result exhibited that some SNP loci, such as rs1015149, rs1062966, and rs11754668, had positive interactions with smoking, which is a risk factor for FLD susceptibility.[18] This finding is consistent with the results of Zhang et al. on the interactions between *GPX-1* polymorphism and smoking in NAFLD and also agrees with the results of Oniki et al.[19] Interestingly, rs1015149 and rs2273068 had negative interaction with smoking in FLD; thus, they are considered “protective factors” (OR = 0.96 and 0.97, respectively). Smoking is a recognized risk factor that is remarkably associated with the occurrence of many diseases. However, the relationship between smoking and FLD is not clear yet.[20-23] In this study, we found that smoking might reduce the risk of FLD of individuals who carry the CT+CC genotype of rs1015149 or the TT+CT genotype of rs2273068. However, the results do not "advocate" smoking to these population for FLD prevention. A more rational explanation for the reduced FLD risk is that compared with individuals who carry other genotypes, people with the CT+CC genotype of rs1015149 or the TT+CT genotype of rs2273068 may be more able to offset the risk of FLD caused by smoking. The same explanation can also be utilized to explain the interaction between rs11754668 and alcohol intake in this study.

No significant additive interaction was observed in this study. This result is in agreement with the result of Zhao et al.[24] In other words, additive interaction may not be remarkable even if the factors studied have substantial multiplicative interaction. In fact, the interaction between multiple factors is based on multiplication and synergism, whereas additive interaction is relatively rare. In the field of medicine, the analysis models for gene–environment, gene–gene, and gene–environment-gene interactions, such as cross-generation analysis,[25] multifactor dimensionality reduction (MDR),[26] and generalized MDR,[27] are based on multiplication. Although substantial multiplicative interaction results were not observed

in the present study, this study still provided a meaningful attempt to explore the gene–environment interaction in FLD, which might be ignored.

Dietary factors are important influencing factors of FLD. Numerous literatures have reported the association between different dietary patterns or food intake and the incidence of FLD. For example, the high intake of meat, high-fat dairy products, and refined grains may increase the risk of FLD, whereas a diet based on fruits, vegetables, whole grains, fish, and olive oil can reduce FLD risk.[28, 29] A cross-sectional study based on Chinese adolescents illustrated that adolescents who have traditional Chinese diet have lower risks of FLD compared with those with Western diet.[30] Therefore, the potential impact of diet on the result needs to be fully considered and controlled to reduce analysis error. In addition, in this study, we ensured data quality and improved the reliability of the results. The advantages are as follows. (1) This study is the first epidemiological study to uncover the associations of *TFEB* polymorphism and gene–environment interaction with FLD. (2) All participants were from the same area with relatively similar genetic background, living environment, and habits; these similarities were helpful to control potential confounding factors. (3) In terms of grouping, gender and age (±3 years) were adopted in matching to reduce the influence of gender and age on the results to a certain extent.

However, this study also has many deficiencies that need to be further improved. First, the sample size is relatively small, and sampling error is difficult to decrease. Second, the conclusions are based on the population from Gongcheng County. Therefore, the applicability of the conclusions to other populations is limited, and extrapolation is deficient. Third, the degree of FLD was not classified. Thus, the effect of research factors on the process of FLD might have been ignored. Finally, the causal demonstration power is not strong because of the case–control design.

Conclusion

The polymorphisms of the rs1015149, rs1062966, rs11754668, rs14063, and rs227306 of the *TFEB* gene are not directly associated with FLD susceptibility, but the risk can be changed through gene–environment interaction.

Abbreviations

FLD: fatty liver disease; TFEB: transcription factor EB; NAFLD: nonalcoholic fatty liver disease; SNPs: single nucleotide polymorphisms; PPARα: peroxisome proliferator-activated receptor alpha; PGC-1α: proliferator-activated receptor gamma coactivator 1 alpha; WC: waist circumference; BMI: body mass index; SBP/DBP: systolic/diastolic blood pressure; HbA1C: glycosylated hemoglobin; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglyceride; TC: total cholesterol; GLU: fasting plasma glucose; ALB: albumin; ALT: alanine aminotransferase; AST aspartate transaminase; UA: uric acid; HWE: Hardy–Weinberg equilibrium; RERI: relative excess risk due to interaction; AP: attributable proportion of interaction; SI: synergy index; DM: Dominant model; RM: Recessive model ; MDR: multifactor dimensionality reduction

Declarations

Acknowledgments:

Not applicable.

Author contributions:

Conception and design: Chunbao Mo, Tingyu Mai and Jiansheng Cai;

Acquisition of data: Haoyu He, Huaxiang Lu, Xu Tang, Quanhui Chen, Xia Xu, Chuntao Nong. Shuzhen Liu, Tan Dechan, Qiumei Liu and Min Xu;

Analysis and interpretation of data: Chunbao Mo, Tingyu Mai, Jiansheng Cai and Haoyu He;

Writing, review, and/or revision of the manuscript: Chunbao Mo, Tingyu Mai, Jiansheng Cai, Li You and Jian Qin;

Administrative, technical, or material support: You Li, Zhiyong Zhang and Jian Qin;

Study supervision: Zhiyong Zhang and Jian Qin. All authors approved the final manuscript.

Funding

This work was funded by National Natural Science Foundation of China [No. 8196120388]; Guangxi Science and Technology Development Project [No. AD17129003]; Guangxi Graduate Education Innovation Project [NO. YCSW2020230]

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Our research protocol was approved by the Ethics Committee of Guilin Medical University.

Consent for publication

Not applicable.

Competing interests

The authors declare that there is no conflict of interests.

Author details

[1] Department of Pathophysiology, Faculty of Basic Medical Sciences, Guilin Medical University，Guilin 541004, Guangxi, China

[2] Department of Environmental Health and Occupational Medicine, School of Public Health, Guilin Medical University, Guilin 541004, Guangxi, China

[3] Department of Environmental and Occupational Health, School of Public Health, Guangxi Medical University, Nanning 530021, Guangxi, China

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Tables

Table 1 Demographic and behavioral characteristics of the study population

Variables		All Participants (n %)	Control group (n %)	Case group (n %)	χ^2/t	*p value*
Gender	Male	321 (56.32)	192 (56.14)	129 (56.58)	0.011	0.918
	Female	249 (43.68)	150 (43.86)	99 (43.42)		
Age (years) #		58.15±12.42	58.50±12.75	57.64±11.93	0.806	0.42
Nation	Han	98 (17.19)	58 (16.96)	40 (17.54)	0.194	0.907
	Yao	447 (78.42)	268 (78.36)	179 (78.51)		
	Zhuang and others	25 (4.39)	16 (4.68)	9 (3.95)		
Marital status	No partner	77 (13.51)	49 (14.33)	28 (12.28)	0.491	0.484
	Have a partner	493 (86.49)	293 (85.67)	200 (87.72)		
Education	Primary school and below	354 (62.11)	223 (65.20)	131 (57.46)	3.49	0.062
	Junior high school and above	216 (37.89)	119 (34.80)	97 (42.54)		
Occupation	Farmer	519 (91.05)	309 (90.35)	210 (92.11)	0.517	0.472
	Others	51 (8.95)	33 (9.65)	18 (7.89)		
Household income (Yuan)	5000	154 (27.02)	88 (25.73)	66 (28.95)	0.718	0.397
	≥5000	416 (72.98)	254 (74.27)	162 (71.05)		
Diabetes	No	546 (95.79)	333 (97.37)	213 (93.42)	5.285	0.022*
	Yes	24 (4.21)	9 (2.63)	15 (6.58)		
Hypertension	No	464 (81.40)	284 (83.04)	180 (78.95)	1.514	0.218
	Yes	106 (18.60)	58 (16.96)	48 (21.05)		
Smoking	No	465 (81.58)	273 (79.82)	192 (84.21)	1.751	0.186
	Yes	105 (18.42)	69 (20.18)	36 (15.79)		
Drinking	No	371 (65.09)	221 (64.62)	150 (65.79)	0.082	0.774
	Yes	199 (34.91)	121 (35.38)	78 (34.21)		
Smoking amount (pack year)#		5.83±16.78	6.37±17.69	5.03±15.31	0.934	0.351
Alcohol intake (g/day)#		17.26±40.40	17.84±43.75	16.39±34.87	0.419	0.675
Strenuous physical activity (h/day)#		1.37±7.17	1.19±6.04	1.65±8.59	-0.743	0.458
Moderate physical activity (h/day)#		4.52±11.32	4.54±11.43	4.49±11.18	0.049	0.961
Daily walking time (h)#		3.27±2.21	3.38±2.24	3.09±2.14	1.526	0.128

Daily sitting time (h)[#]	3.64±1.79	3.50±1.76	3.84±1.83	-2.212	0.027*

[#]Mean ± SD; *$p < 0.05$ was considered statistically significant.

Table 2 Daily food intake of the participants

Food category	All Participants	Control group	Case group	*t*	*p value*
Cereals and their products	263.80±161.91	262.63±168.13	265.55±152.47	-0.211	0.833
Potatoes	38.56±86.34	39.72±91.08	36.82±78.86	0.392	0.695
Vegetables	313.68±315.28	325.79±336.87	295.53±279.52	1.123	0.262
Fruits	254.53±269.71	256.25±282.77	251.94±249.43	0.187	0.852
Beans and their products	36.04±50.25	36.04±51.40	36.04±48.59	-0.001	0.999
nuts	13.40±26.02	12.69±23.49	14.47±29.42	-0.803	0.422
Meat and poultry	83.37±88.86	87.23±94.64	77.58±79.25	1.271	0.204
Fish and aquatic products	17.88±29.63	17.28±29.14	18.77±30.39	-0.585	0.559
Milk and their products	35.78±36.31	34.85±35.59	37.18±37.40	-0.752	0.452
Eggs and their products	33.23±71.65	34.74±76.36	30.96±64.04	0.617	0.538
Cooking oil	35.00±26.66	35.71±27.58	33.94±25.23	0.776	0.438
Salt	9.60±7.51	9.93±8.24	9.11±6.24	1.276	0.202

Note: Data are expressed as mean ± SD. The unit for each food category is gram.

Table 3 Clinical indicators of the study population

Clinical indicators	All Participants	Control group	Case group	*t*	*p value*
WC	81.39±10.49	76.78±9.14	88.30±8.40	-15.23	<0.001**
BMI	24.55±17.59	21.99±3.44	28.40±27.07	-3.558	<0.001**
SBP	136.02±24.23	134.39±23.15	138.47±25.63	-1.972	0.049*
DBP	82.52±15.14	80.72±13.59	85.22±16.88	-3.512	<0.001**
HbA1C	5.96±1.06	5.82±0.90	6.17±1.25	-3.595	<0.001**
LDL-C	3.44±0.98	3.30±0.94	3.63±0.99	-4.026	<0.001**
HDL-C	1.69±0.39	1.76±0.39	1.58±0.37	5.559	<0.001**
TC	5.56±1.05	5.40±1.03	5.80±1.03	-4.533	<0.001**
TG	1.60±1.61	1.21±1.28	2.18±1.86	-6.875	<0.001**
GLU	5.07±1.53	4.87±1.24	5.37±1.85	-3.543	<0.001**
ALB	44.06±2.34	43.77±2.34	44.50±2.29	-3.67	<0.001**
ALT	21.90±12.99	19.29±12.29	25.81±13.06	-5.976	<0.001**
AST	24.09±11.27	23.55±10.91	24.91±11.77	-1.415	0.158
UA	333.34±100.58	306.72±91.26	373.28±100.87	-8.175	<0.001**

Note: Data are expressed as mean ± SD; $^{*}p < 0.05$ and $^{**}p < 0.001$ were considered statistically significant; WC: waist circumference (cm), BMI: body mass index, SBP/DBP: systolic/diastolic blood pressure (mmHg), HbA1C: glycosylated hemoglobin (%), LDL-C: low-density lipoprotein cholesterol (mmol/L), HDL-C: high-density lipoprotein cholesterol (mmol/L), TG: triglyceride (mmol/L), TC: total cholesterol (mmol/L), GLU: fasting plasma glucose (mmol/L), ALB: albumin (g/L), ALT: alanine aminotransferase (U/L), AST aspartate transaminase (U/L), UA: uric acid (μmol/L).

Table 4 Descriptive statistics of *TEFB* genotypes

SNPs	Alleles/Genotypes	Control group (n %)	Case group (n %)	χ^2	*p value*
rs1015149	C	390 (0.57)	267 (0.59)	0.210	0.647
	T	292 (0.43)	189 (0.41)		
	CC	114 (33.33)	78 (34.21)	1.018	0.797
	CT	162 (47.37)	111 (48.68)		
	TT	65 (19.01)	39 (17.11)		
rs1062966	C	550 (0.81)	369 (0.81)	0.004	0.947
	T	128 (0.19)	85 (0.19)		
	CC	221 (64.62)	147 (64.47)	0.727	0.867
	CT	108 (31.58)	75 (32.89)		
	TT	10 (2.92)	5 (2.19)		
rs11754668	C	624 (0.92)	406 (0.89)	2.404	0.121
	G	56 (0.08)	50 (0.11)		
	CC	286 (83.63)	181 (79.39)	3.828	0.281
	GC	52 (15.20)	44 (19.30)		
	GG	2 (0.58)	3 (1.32)		
rs14063	G	463 (0.68)	312 (0.69)	0.036	0.849
	A	213 (0.32)	140 (0.31)		
	AA	28 (8.19)	19 (8.33)	0.208	0.976
	AG	157 (45.91)	102 (44.74)		
	GG	153 (44.74)	105 (46.05)		
rs2273068	C	590 (0.87)	406 (0.89)	1.806	0.179
	T	90 (0.13)	48 (0.11)		
	CC	254 (74.27)	183 (80.26)	3.675	0.299
	CT	82 (23.98)	40 (17.54)		
	TT	4 (1.17)	4 (1.75)		

Table 5 Logistic regression analysis between *TEFB* polymorphism and FLD

Genotype	*β*	*S.E.*	*Wald χ²*	*p* value	*OR*	95%*CI*
rs1015149						
CC			0.376	0.829	1.000	
CT	-0.009	0.192	0.002	0.962	0.991	0.68~1.44
TT	-0.142	0.251	0.321	0.571	0.868	0.53~1.42
Dominant model TT+CT vs. CC	0.046	0.181	0.063	0.802	1.047	0.73~1.49
Recessive model TT vs. CT+CC	0.137	0.224	0.373	0.541	1.146	0.74~1.78
rs1062966						
CC			0.364	0.834	1.000	
CT	0.049	0.185	0.071	0.789	1.051	0.73~1.51
TT	-0.284	0.559	0.258	0.612	0.753	0.25~2.25
Dominant model TT+CT vs. CC	-0.025	0.180	0.019	0.889	0.975	0.69~1.39
Recessive model TT vs. CT+CC	0.300	0.555	0.293	0.589	1.350	0.45~4.01
rs11754668						
CC			2.507	0.286		
GC	0.293	0.226	1.678	0.195	1.340	0.86~2.09
GG	0.888	0.919	0.933	0.334	2.430	0.40~14.73
Dominant model GG+GC vs. CC	-0.322	0.221	2.120	0.145	0.725	0.47~1.12
Recessive model GG vs. GC+CC	0.322	0.221	2.120	0.145	1.380	0.89~2.13
rs14063						
AA			0.150	0.928		
AG	0.003	0.326	0.000	0.992	1.003	0.53~1.90
GG	-0.066	0.180	0.136	0.712	0.936	0.66~1.33
Dominant model AA+AG vs. GG	0.056	0.173	0.104	0.747	1.057	0.75~1.48
Recessive model AA vs. AG+GG	-0.037	0.314	0.014	0.906	0.964	0.52~1.78
rs2273068						
CC			3.896	0.143		
CT	-0.413	0.218	3.605	0.058	0.662	0.43~1.01
TT	0.297	0.715	0.173	0.678	1.346	0.33~5.47
Dominant model TT+CT vs.CC	0.366	0.211	3.009	0.083	1.442	0.95~2.18
Recessive model TT vs. CT+CC	-0.389	0.713	0.297	0.586	0.678	0.17~2.74

Table 6 Results of gene–environment multiplication and additive interactions (Only the parts with statistical significance are exhibited)

Gene-environment interaction		*β*	*p* value	*OR* (95%*CI*)	RERI (95%*CI*)	AP (95%*CI*)	SI (95%*CI*)
rs1015149	RM × Smoking amount	-0.037	0.032*	0.960 (0.930,1.000)	0.003 (-0.025~0.031)	0.002 (-0.017~0.021)	1.007 (0.947~1.072)
rs1062966							
	DM × Smoking amount	0.027	0.035*	1.030 (1.000,1.050)	0.000 (-0.015~0.015)	0.000 (-0.018~0.018)	1.000 (0.924~1.083)
	DM×BMI	0.270	<0.001**	1.310 (1.140,1.510)	-0.256 (-0.374~-0.138)	-121.1 (-509.0~266.7)	1.345 (1.145~1.579)
rs11754668							
	DM × Smoking amount	0.032	0.039*	0.970 (0.940,1.000)	-0.007 (-0.021~0.007)	-0.008 (-0.025~0.010)	1.067 (0.842~1.352)
	DM × Alcohol intake	-0.017	0.037*	0.980 (0.970,1.000)	-0.006 (-0.020~0.007)	-0.007 (-0.022~0.009)	1.098 (0.600~2.010)
	RM × Smoking amount	0.032	0.039*	1.030 (1.000,1.060)	-0.007 (-0.025~0.011)	-0.006 (-0.021~0.008)	0.946 (0.771~1.162)
	RM × Alcohol intake	0.017	0.037*	1.020 (1.000,100.030)	-0.006 (-0.021~0.009)	-0.006 (-0.019~0.008)	0.927 (0.572~1.501)
rs2273068							
	DM × Smoking amount	-0.028	0.024	0.970 (0.950,1.000)	0.000 (-0.029~0.029)	0.000 (-0.017~0.017)	1.000 (0.961~1.041)

Note: DM: Dominant model; RM: Recessive model; * $p < 0.05$ and ** $p<0.001$ were considered as statistically significant

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