Letter to the Editor

Interaction Effects of Gene Polymorphisms and Obesity on Nonalcoholic Fatty Liver Disease*

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Nonalcoholic fatty liver disease (NAFLD) is a common chronic condition that affects adults and has a global prevalence of 25%[1], with the prevalence being 31% among the general population in China[2]. A meta-analysis of studies conducted over the past few years has reported that the predicting factors for NAFLD include obesity, type 2 diabetes mellitus, lifestyle, and genetic variants. The risk of developing NAFLD has been reported to be 3.5-fold higher in individuals with obesity than in the general population[3]. Furthermore, several gene polymorphisms have been identified as potential candidates for determining the risk of developing NAFLD[4], including rs738409 in *pat*atin-like phospholipase domain-containing 3 (*PNP*LA3) gene, *SAM*M50 gene polymorphisms (rs2143571), *Par*vin Beta (*PAR*VB) gene polymorphisms (rs6006611, rs6006473, and rs5764455), *APO*E gene polymorphisms (rs429358 and rs7412), *phos*phatidylethanolamine N-methyl transferase gene V175M, *lep*tin receptor (LEPR) gene K109R polymorphism, and *Kru*ppel-like factor 6 gene polymorphism KLF6-IVS1-27G > A. Another earlier study reported that both genes and environmental predictors play a major role in the risk of developing NAFLD, and despite their higher prevalence in obese individuals, the disease may affect nonobese subjects[5]. However, the interaction effects of obesity and genes on NAFLD have not yet been investigated among the Chinese population.

This study was conducted to investigate the interaction effects of obesity and genes using a multifactor dimensionality reduction (MDR) model. The study sample included subjects belonging to the Chinese Hui ethnicity acquired from the Ningxia Hui Autonomous Region. The inclusion criteria were (1) permanent residency in the local community, (2) age ≥ 55 years, and (3) no history of alcoholism. Subjects who were unable to complete the survey due to vision or hearing disabilities or had a history of serious illness such as cancer or stroke were excluded. A total of 1,856 community residents were eligible for inclusion in the study. Trained interviewers conducted in-person surveys when the participants attended a health examination at community health care settings.

All participants underwent abdominal B-ultrasonography examination, a commonly used noninvasive method for evaluating the different pathological processes of NAFLD[6]. Details regarding body mass index (BMI) and fasting plasma glucose (FPG) levels were obtained from the health check records. A standard questionnaire was used to collect details related to sociodemographic information, including age, gender, living situation (living alone or not), and education level (categorized as illiterate, primary school, junior school, senior school, and above). Genotypes were detected by the Mass ARRAY system (Sequenom, San Diego, CA, USA) using chip-based, matrix-assisted laser desorption ionization time-of-flight mass spectrometry technology performed at a genetic testing company in China (BGI-Shenzhen). Chi-squared analysis was performed to determine the Hardy–Weinberg equilibrium (APOE: $\chi^2 = 0.30$, $P = 0.224$; rs738409: $\chi^2 = 0.66$, $P = 0.416$; rs2143571: $\chi^2 = 0.60$, $P = 0.438$; rs6006611: $\chi^2 = 1.20$, $P = 0.273$). This study was approved by the Institutional Review Board at Ningxia Medical University (document number: 2015151), and written informed consent was obtained from all study participants.

As shown in Table 1, a total of 1,476 participants with an average age of 63.3 (Sd = 4.6) years who completed the questionnaire had the laboratory data available that were included in the final analysis; more than half of the participants (767,

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52.0%). were females. A total of 341 (23.10%) participants met the criteria of NAFLD according to the guidelines for the diagnosis and treatment of NAFLDs developed by the Fatty Liver and Alcoholic Liver Disease Study Group of the Chinese Liver Disease Association[2]. A strong association was found between obesity and the risk of developing NAFLD, which was consistent with the majority of studies reported since the past decade. The proportions of obesity and fasting blood glucose abnormalities were higher in the NAFLD group than in the control group ($P < 0.05$).

Regarding the genotype frequencies, there were statistically significant differences in the rs429358/ rs7412 genotype frequency between cases and control subjects ($\chi^2 = 11.35$, $P = 0.035$) (Table 2). There was a lower proportion of ε4 carriers in the NAFLD group than in the control group ($\chi^2 = 6.73$, $P = 0.009$). The rs738409 genotype frequency was significantly different between the NAFLD group and the control group ($\chi^2 = 8.98$, $P = 0.032$). However, there was no statistical association between the

Table 1. Demographic characteristics of the participants

Variables	Total (*n* = 1,476)	NAFLD (*n* = 341)	Controls (*n* = 1,135)	t/x^2	*P* value
Age, mean ± SD, years	63.3 ± 4.6	63.1 ± 4.4	63.4 ± 4.7	1.20	0.229
Gender, male, *n* (%)	767 (52.0)	174 (51.0)	593 (52.2)	0.16	0.692
Education, *n* (%)					
Illiterate	841 (57.0)	185 (54.3)	656 (57.8)	6.16	0.104
Primary school	366 (24.8)	83 (24.3)	283 (25.0)		
Junior school	183 (12.4)	44 (13.0)	139 (12.3)		
Senior school	86 (5.9)	29 (8.5)	57 (5.0)		
Living status, alone, *n* (%)	189 (12.8)	50 (14.7)	139 (12.2)	1.37	0.242
Obesity, yes, *n* (%)	245 (16.6)	126 (36.7)	119 (10.5)	132.06	< 0.001
FPG, > 6.1 mmol/L, *n* (%)	193 (13.1)	63 (18.5)	130 (11.5)	11.38	0.001

Note. NAFLD: nonalcoholic fatty liver disease; FPG: fasting plasma glucose.

Table 2. Allele and genotype distributions of SNPs and association between each SNP and NAFLD

SNPs	Sample	*N*	Genotype						χ^2	*P*	Allele		χ^2	*P*	*OR* (95% *CI*)
rs429358/ rs7412			ε3/ε3	ε4/ε4	ε3/ε4	ε3/ε2	ε2/ε2	ε2/ε4			ε4	ε3/ε2			
	case	341	223	1	51	60	2	4			53	629			
	control	1,135	729	15	225	141	5	20	11.35	0.035	255	2,015	6.73	0.009	0.67 (0.49–0.91)
rs738409			C/C	C/G	G/G						G	C			
	case	341	131	150	59						268	412			
	control	1,135	460	535	140				8.98	0.032	815	1,455	2.78	0.096	1.16 (0.98–1.39)
rs2143571			G/G	G/A	A/A						A	G			
	case	341	143	151	47						245	437			
	control	1,135	470	530	135				1.11	0.581	800	1,470	0.17	0.744	1.03 (0.86–1.23)
rs6006611			G/G	G/A	A/A						G	A			
	case	341	75	173	93						323	359			
	control	1,135	275	548	311				1.18	0.760	1,098	1,170	0.23	0.630	0.96 (0.81–1.13)

Note. *OR*: odds ratio; *CI*: confidence interval.

rs6006611 genotype frequency and the risk of developing NAFLD, which was inconsistent with the finding of the previous study[4]. This could be attributed to several possible explanations, including the different criteria used for diagnosing NAFLD and the genetic background differences between Chinese Hui and Han ethnicities. Xu et al. had reported that genetic variations exist between Chinese Hui people and Chinese Han people in the susceptibility of vitamin D receptor polymorphisms related type 2 diabetes mellitus, and in the T-cell immunoglobulin and *muc*in domain 4 genes (rs7700944) frequency distribution[7].

As depicted in Table 3, the MDR analysis demonstrated a statistically significant interaction between obesity and rs6006611 ($P < 0.001$), with a testing balance accuracy of prediction of 52.68% and a CVC of 8/10. The interaction dendrogram is shown in Supplementary Figure S1 (available in www.besjournal.com). This significant interaction between obesity and rs6006611 persisted even after controlling for the demographic variables (age, gender, FPG level, and education level) using an unconditional logistic regression model ($\beta_{G\times E} = 1.63$, $OR_{G\times E} = 4.97$, 95% *CI* = 3.59–6.89, $P < 0.001$). This finding was consistent with a previous study conducted in a varied population, in which a potential interaction effect was detected between rs266729 and rs822393 on the risk of developing NAFLD using the MDR model (CVC = 10/10, testing accuracy = 0.6217, $P = 0.001$)[8]. In addition, another study reported the presence of a significant interaction between cytochrome genes and glutathione S-transferase genes, which resulted in a significant reduced risk of developing NAFLD among subjects carrying the two unfavorable gene variants, and the study also found a significant interaction effect between rs738409 and weight gain (of ≥ 10 kg after age 20 years) on the pathogenesis of NAFLD[9].

The *PAR*VB gene is involved in lipid accumulation and/or fibrosis in the liver, resulting in the development of NAFLD. Obesity can cause hepatic steatosis through endotoxin or oxidative stress damage, as well as excessive secretion of cytokines (include tumor necrosis factor-α, interleukin-6) that induced by the accumulation of liver fat[10]. Therefore, obese individuals who are rs6006611 G allele carriers have an increased tendency to accumulate fat in the liver.

To summarize, we found that the interaction effects between polymorphisms in rs6006611 and obesity were associated with the development of NAFLD. These findings could provide a reference for health management institutions to better estimate the social burden of obesity on the health of the population. These findings are also helpful to understand the complex interactions between obesity and susceptible gene variants of NAFLD; furthermore, the results could provide a theoretical basis for developing precise disease prevention and treatment strategies. Nevertheless, a further longitudinal study is needed to confirm the findings due to the limitations of the present study, which includes the cross-sectional data that provided limited evidence of the causal temporal paths in the model and also the fact that genetic exposure could have been measured as an unchangeable variable. In addition, due to the weak effects of the SNPs on the outcomes, there may be a risk of failure to detect the potential association between the gene variants and NAFLD risk under the limited sample size. Moreover, regarding feasibility, a noninvasive method was used in this study to evaluate the disease without considering the severity of NAFLD in the final data analysis, which might lead to information bias.

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Table 3. The best model analyzing gene variants and obesity for predicting the prevalence of NAFLD

Models	Training accuracy (%)	Testing accuracy (%)	CVC	χ^2	*P* value	*OR* (95% *CI*)
FPG	53.79	51.11	5/10	5.51	0.018	1.78 (1.28–2.48)
Obesity, rs6006611	55.41	52.68	8/10	11.38	< 0.001	1.62 (1.24–2.11)
FPG, rs738409, rs6006611	56.39	50.60	5/10	15.43	< 0.001	1.68 (1.31–2.15)

Note. FPG: fasting plasma glucose; CVC: cross-validation consistency; *OR*: odds radio; *CI*: confidence interval.

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Supplementary Figure S1. Interaction dendrogram of genes and obesity. The red line represents a synergistic interaction (obesity and rs6006611), the orange line represents a weaker synergistic interaction (rs738409 and rs6006611), and the blue line represents a redundant interaction (FPG and rs 6006611).