Hepat Mon. 2015 December; 15(12): e33155. doi: 10.5812/hepatmon.33155

Published online 2015 December 19. **Research Article**

Association Between *LYPLAL1* rs12137855 Polymorphism With Ultrasound-Defined Non-Alcoholic Fatty Liver Disease in a Chinese Han Population

Chen Yuan,[1] Linlin Lu,[2,3] Baiquan An,[1,4] Wenwen Jin,[4] Quanjiang Dong,[3,4] Yongning Xin,[1,2,4,*] and Shiying Xuan[1,*]

[1]Department of Gastroenterology, Qingdao Municipal Hospital, Nanjing Medical University, Qingdao, China
[2]Digestive Disease Key Laboratory of Qingdao, Qingdao, China
[3]Central Laboratories, Qingdao Municipal Hospital, Qingdao, China
[4]Department of Gastroenterology, Qingdao Municipal Hospital, Qingdao, China

*Corresponding Authors: Shiying Xuan, Department of Gastroenterology, Qingdao Municipal Hospital, Nanjing Medical University, Qingdao, China. Tel: +86-53288905289, Fax: +86-53288905293, E-mail: xuansydxy@163.com; Yongning Xin, Department of Gastroenterology, Qingdao Municipal Hospital, Nanjing Medical University, Qingdao, China. Tel: +86-53288905289, Fax: +86-53288905293, E-mail: xinyongning@163.com

Received 2015 September 14; **Revised** 2015 October 9; **Accepted** 2015 October 17.

Abstract

Background: Recent genome-wide association studies (GWAS) identified that gene Lysophospholipase-like 1 (*LYPLAL1*) rs12137855 associated with non-alcoholic fatty liver disease (NAFLD). No research has been performed regarding the association between *LYPLAL1* and NAFLD in China.

Objectives: The aim of the present study was to investigate the association between the gene *LYPLAL1* rs12137855 and NAFLD, and the effect on serum lipid profiles in a Chinese Han population.

Patients and Methods: *LYPLAL1* rs12137855 gene was genotyped in 184 patients with NAFLD and 114 healthy controls using sequencing and polymerase chain reaction analysis (PCR). We tested serum lipid profiles using biochemical methods.

Results: No significant differences in genotype and allele frequencies of *LYPLAL1* rs12137855 was found between the NAFLD group and the controls group ($P > 0.05$). Subjects with the variant *LYPLAL1* rs12137855 CC genotype had a higher mean weight, body mass index (BMI) and low density lipoprotein (LDL).

Conclusions: Our results showed for the first time that *LYPLAL1* gene is not associated with a risk of NAFLD development in the Chinese Han population. The variant carriers of overall subjects significantly increased weight, BMI and LDL.

Keywords: Single Nucleotide Polymorphism, Non-Alcoholic Fatty Liver Disease, *LYPLAL1*

1. Background

Non-alcoholic fatty liver disease (NAFLD), the hepatic manifestation of metabolic syndrome, includes a spectrum of diseases ranging from simple steatosis, through steatohepatitis (NASH), to fibrosis and ultimately cirrhosis (1, 2). The disease definition and modalities used for diagnosis and epidemiology studies are not standardized (3). The prevalence of NAFLD increased rapidly and it affects about 20% to 30% of the population in Western countries (4) and 15% in China (5). As a lipid metabolism disorder, NAFLD has a strong genetic component. A recent GWAS from The genetics of obesity-related liver disease consortium identified *LYPLAL1* rs12137855 for NAFLD in 7177 adults of European ancestry (6).

LYPLAL1 encodes lysophospholipase-like protein 1, a 26 kDa cytosol protein, which belongs to a subclass of lysophospholipase family (7). Recently, several single nucleotide polymorphisms (SNPs), near human *LYPLAL1* gene were revealed to be significantly associated with fat distribution in a relatively sex-specific pattern (8), such as 3 SNPs including rs4846567 (9), rs2605100 (10) and rs2820443 (11) near *LYPLAL1* gene, which are associated with increased waist-hip ratio (WHR) adjusted for BMI only in women not men. SNP rs11118316 at *LYPLAL1* is associated with visceral adipose tissue/subcutaneous adipose tissue ratio in both men and women (12), and SNP at rs12137855 near *LYPLAL1* gene is strongly associated with NAFLD (13). Subsequent studies showed that *LYPLAL1* plays an important role in fat distribution and lipid metabolism. *LYPLAL1* rs12137855, as the susceptibility gene of

NAFLD, was widely studied, but the results were inconsistent. No research has been performed on the association between polymorphism of *LYPLAL1* and NAFLD in Chinese Han population.

2. Objectives

The aim of our study was to investigate the association between NAFLD and *LYPLAL1* in Chinese Han population and assess the effect of this gene on serum lipid profiles.

3. Patients and Methods

3.1. Subjects

The study was performed in accordance with the principles of declaration of Helsinki and its appendices (14). This study was approved by the ethical committee of Qingdao municipal hospital (Qingdao, China) and a written informed consent form was obtained from all patients before participation in the study.

From May 2010 to May 2014, we selected a total of 298 unrelated adult subjects, including 184 unrelated Chinese patients of both genders and different ages (85 males, 97 females, mean age 43.18 ± 11.53 years) diagnosed with NAFLD and 114 healthy controls matched for sex and age (57 males, 57 females, mean age 40.77 ± 11.47 years) by B-type ultrasonography (15). The subjects were collected from the department of gastroenterology and the medical center of Qingdao municipal hospital. All subjects were unrelated and ethnically Han Chinese origin. The diagnosis of NAFLD was performed under standard clinical evaluation conditions according to the AASID criteria. Other causes of liver disease were excluded, including increased alcohol intake (> 210/140 g/wk for males/females), as confirmed by at least one family member or friend and carboxydesialylated transferrin determination, viral and autoimmune hepatitis, hereditary hemochromatosis, and alphal-antitrypsin deficiency (16). We excluded other related disease, such as subjects with type 1 diabetes mellitus and coronary atherosclerotic disease (CAD). The controls were confirmed as healthy by medical history, general examinations and laboratory examinations at the same hospital.

3.2. Biochemical Analyses

Blood samples of each subject for biochemical analyses were collected into ethylene diamine tetraacetic acid-containing tubes after an 12-hour overnight fast and the following information for each subject was gathered; height, body mass, waist, hip circumference, calculating body mass index (BMI) equals to mass (kg)/ height $(m)^2$. Environmental factors, such as diet and physical activity, were not recorded in this study. The blood sample tested for total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) using routine enzymatic methods. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyltransferase (GGT) concentrations were measured as previously described (17).

3.3. Genetic DNA Extraction and Genotyping

The genomic DNA purification kit (BioTeke, Biotechnology, Beijing, China) was used for extracting DNA from peripheral blood following the manufacturer's instructions and stored at −20°C until use. Genotyping for *LYPLAL1* (rs12137855) was performed by polymerase chain reaction (PCR) analysis using the following primers for *LYPLAL1* polymorphism: 5'-TCCTAAGTTCCTATTGTCCCTTCA-3' and 5'-TGCTGTGGGGTGAGTCA-3'. PCR amplification (Labnet, United States) was performed as follows; initial step of 95°C for 10 minutes, followed by 35 cycles; denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute and elongation at 70 °C for 1 minute. All PCR products were resolved using 2% agarose gel electrophoresis at 110 V for 30 minutes with a 237-base pair product in size. The *LYPLAL1* genotypes were detected by direct DNA sequencing using the ABI Prism sequence detection system ABI3730 (Foster city, CA, USA). The genotyping call rate was more than 95% and the completion rate was > 99%. Genotyping was performed in a blinded fashion.

3.4. Statistical Analysis

Statistical analyses were performed using SPSS statistical software, version 17.0 for window (SPSS Inc. Chicago, IL, USA). Hardy-Weinberg equilibrium between expected and observed genotype distributions was assessed using the χ^2 test. Genotype and alleles were estimated by chi-square test and DNA distributions between NAFLD patients and controls were analyzed by Pearson's χ^2 test or Fisher's exact test where appropriate. The baseline characteristics of participants shown as mean ± SD. Differences in characteristics between different groups were examined using student's t test, paired samples t-test or χ^2 test. The strength of the association between the polymorphism and NAFLD was evaluated by logistic regression analysis adjusted for confounders (age, sex, smoking and hypertension, which were considered as continuous variables). Level of significance was defined as $P < 0.05$.

4. Results

4.1. Characteristics of the Study Population

The clinical characteristics of the study participants are shown in Table 1.

4.2. LYPLAL1 rs12137855 Genotypes and Allele Distribution

The genotypes distribution of *LYPLAL1* was in accordance with the Hardy-Weinberg equilibrium in NAFLD and control groups (P_{NAFLD} = 0.323; $P_{control}$ = 0.230, respectively). To ensure the accuracy of our genotyping, we randomly repeated DNA sequencing in 100 subjects for reverse sequencing. The success rate of duplicated genotyping was more than 100%. The genotype and allele distribution are shown in Table 2, which indicates no significant difference between the two groups (P > 0.05). The gene *LYPLAL1* did not increase the risk of developing NAFLD (OR = 0.622, 95% CI: 0.334 - 1.159).

4.3. LYPLAL1 rs12137855 Association with Clinical Parameters in Non-Alcoholic Fatty Liver Disease Patients

To explore whether gene polymorphism affect the laboratory parameters, we compared non-carriers and carriers of variant allele (rs12137855) in all subjects, NAFLD patients and healthy controls, respectively (Table 3); the results showed that there was a significant difference in weight, BMI and LDL.

Table 1. Demographics and Clinical Characteristics of Patients With NAFLD and Controls[a]

Characteristics	NAFLD Patients (n = 184)	Controls (n = 114)	P Value
Height, cm	167.72 ± 8.23	165.96 ± 6.44	.053
Weight, kg	74.29 ± 11.34	63.55 ± 10.88	.000
BMI, kg/m^2	26.34 ± 3.05	23.04 ± 3.47	.000
Waist circumference, cm	92.36 ± 9.17	82.28 ± 8.79	.000
Hip circumference, cm	102.91 ± 8.22	96.92 ± 9.13	.000
ALT, U/L	25.43 ± 14.17	19.39 ± 10.14	.000
AST, U/L	21.91 ± 11.11	19.48 ± 6.34	.034
GGT, U/L	21.35 ± 8.613	14.96 ± 5.731	.000
Glu, mmol/L	5.60 ± 1.88	5.01 ± 1.24	.003
TG, mmol/L	1.76 ± 0.95	1.13 ± 0.67	.000
TC, mmol/L	4.90 ± 0.96	4.63 ± 0.93	.018
HDL, mmol/L	1.29 ± 0.48	1.47 ± 0.33	.000
LDL, mmol/L	3.29 ± 0.93	2.93 ± 0.81	.001

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyl transpeptidase; Glu, glucose; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NAFLD, non-alcoholic fatty liver disease patients; TC, total cholesterol; TG, triglyceride.
[a]Data are presented as mean ± SD.

Table 2. Association of Variants in *LYPLAL1* Gene with Risk of NAFLD[a]

SNP (Rs12137855)	NAFLD Patients (n)	Controls (n)	χ^2	P Value
Genotypes			2.261	.133
CC	159	91		
CT	25	23		
Alleles			2.063	.151
C	343	205		
T	25	23		

Abbreviations: NAFLD, non-alcoholic fatty liver disease patients.
[a]P: NAFLD patients vs. control.

Table 3. Clinical Characteristics of *LYPLAL1* (rs12137855 C/C) Carriers and Non-Carriers in the Study Population[a]

Characteristic	Overall Series			NAFLD Patients			Controls		
	Carriers (n = 250)	Non-Carriers (n = 48)	P	Carriers (n = 159)	Non-Carriers (n = 25)	P	Carriers (n = 91)	Non-Carriers (n = 23)	P
Age, y	42.79 ± 11.61	39.52 ± 10.94	.073	43.49 ± 11.59	41.24 ± 11.18	.366	41.56 ± 11.60	37.65 ± 10.61	.145
Female/male	139/111	28/20	.727	90/69	16/9	.487	55/36	18/5	.122
Height, cm	167.15 ± 7.61	166.54 ± 7.82	.616	167.94 ± 8.08	166.36 ± 9.22	.375	165.76 ± 6.53	166.74 ± 6.14	.519
Weight, Kg	70.86 ± 12.06	66.67 ± 13.16	.031	74.66 ± 11.23	71.96 ± 12.03	.270	64.22 ± 10.53	60.91 ± 12.07	.194
BMI, kg/m^2	25.29 ± 3.51	23.96 ± 3.82	.018	26.42 ± 3.14	25.85 ± 2.45	.386	23.32 ± 3.25	21.91 ± 4.03	.078
ALT, U/L	23.07 ± 13.20	23.38 ± 12.65	.884	25.42 ± 14.66	25.52 ± 10.87	.973	18.98 ± 8.88	21.04 ± 14.21	.385
AST, U/L	20.96 ± 9.91	21.06 ± 8.10	.948	22.05 ± 11.83	21.00 ± 4.44	.662	19.07 ± 4.54	21.13 ± 10.89	.383
GGT, U/L	18.83 ± 8.031	17.92 ± 7.930	.471	21.43 ± 8.617	19.60 ± 8.026	.320	14.56 ± 4.949	16.09 ± 7.573	.367
Glu, mmol/L	5.43 ± 1.78	5.09 ± 1.07	.206	5.63 ± 1.94	5.38 ± 1.41	.527	5.07 ± 1.38	4.78 ± 0.31	.326
TG, mmol/L	1.71 ± 1.171	1.57 ± 1.65	.592	1.94 ± 1.72	2.08 ± 2.12	.709	1.32 ± 1.62	1.01 ± 0.53	.372
TC, mmol/L	4.83 ± 0.93	4.60 ± 1.06	.127	4.92 ± 0.91	4.78 ± 1.23	.493	4.69 ± 0.97	4.42 ± 0.76	.216
HDL, mmol/L	1.36 ± 0.44	1.38 ± 0.35	.804	1.29 ± 0.48	1.27 ± 0.31	.862	1.47 ± 0.32	1.48 ± 0.36	.903
LDL, mmol/L	3.20 ± 0.90	2.91 ± 0.84	.040	3.32 ± 0.91	3.09 ± 0.98	.260	2.99 ± 0.84	2.71 ± 0.62	.134

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyl transpeptidase; Glu, glucose; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NAFLD, non-alcoholic fatty liver disease patients; TC, total cholesterol; TG, triglyceride.
[a]Data are Presented as Mean ± SD.

5. Discussion

Speliotes confirmed that *LYPLAL1* is a susceptibility gene of NAFLD (13). In the recent years, this gene has been widely studied, but the results were inconsistent (6, 18). Speliotes et al. observed significant associations with both histologic NAFLD and CT NAFLD at variants or near *LYPLAL1* (6). Our study for the first time investigated the association between *LYPLAL1* rs12137855 and NAFLD in Chinese Han population; we selected 184 NAFLD patients and 114 controls to observe the association between *LYPLAL1* rs12137855 and NAFLD; however, we did not find significant association between gene and NAFLD, which is in accordance with some previous findings (18-23).

Multiple factors are involved in development and progression of NAFLD such as insulin resistance, obesity and oxidative stress (24). In our study, we diagnosed NAFLD using routine blood testing and liver ultrasonography. Lack of direct measurement of hepatic fat content by gold standard liver biopsy reduced the accuracy of diagnosis, but we observed that metabolism indicators change obviously. ALT and AST are used as markers of liver fat accumulation (25-27) and commonly used in clinical practice (28). We can observe significant differences in plasma concentrations of these transaminases between NAFLD and healthy controls. On the contrary, independent of genetic variation in *LYPLAL1*, no difference was reported similar to other biochemical markers. These results were not in accordance with Paola Leon-Mimila and Speliotes study; they confirmed that *LYPLAL1* rs12137855 was associated with increased TG content (6, 29). Speliotes et al. study found similar results with ours. The mechanism is unknown. Interestingly *LYPLAL1*-related proteins have been predicted to play a role in consecutive steps in triglyceride breakdown (30, 31). *PNPLA3* has been confirmed to increase hepatic steatosis through preventing the breakdown of triglyceride (32). Whether *LYPLAL1* has the same function and knowing the mechanism of triglyceride breakdown need more investigations.

Obesity is the major risk factor for NAFLD. Approximately 95% of morbidly obese individuals develop NAFLD (33). For Chinese subjects, BMI of 28 kg/m^2 or more is an index of obesity (16). In this study, BMI was higher in the NAFLD group (26.34 ± 3.06 kg/m^2) than controls (23.04 ± 3.47kg/m^2) ($P < 0.05$), also higher in the variant carrier (25.29 ± 3.51 kg/m^2) than non-carrier (23.96 ± 3.82 kg/m^2) controls in all subjects ($P < 0.05$). Independent of this gene in NAFLD group and control group the difference did not reach statistical significant. We observed that BMI of carriers was greater than non-carriers. Our study suggested that *LYPLAL1* can influence BMI, which reflects the association with obesity indirectly. Our findings for the first time found a significant difference between variant carriers and non-carriers regarding LDL; the mechanism is not clear and needs further research.

As far the studies on Asian population were negative (18, 19), and we have reasons to doubt the correlation of the gene with NAFLD in Asian population. To obtain more precise results, larger studies on multiple ethnic groups, such as Asian Indian or Korean should be performed. Our

results may also be due to small sample size, ethnic differences in linkage disequilibrium (LD) patterns, ethnic-specific association and gene/environment interactions.

This study provided preliminary evidence that there is no association between *LYPLAL1* rs12137855 polymorphism and development of NAFLD in Chinese Han origin for the first time. The C allele of the rs12137855 significantly affects weight, BMI and LDL. Further studies with large study samples and different ethnicity are needed to investigate the influence of this gene on NAFLD.

Footnotes

Authors' Contribution: Study concept and design: Chen Yuan, Linlin Lu; acquisition of data: Chen Yuan, Linlin Lu, Baiquan An, and Wenwen Jin; analysis and interpretation of data: Chen Yuan, and Quanjiang Dong; drafting of the manuscript: Chen Yuan, Yongning Xin; critical revision of the manuscript for important intellectual content: Shiying Xuan; statistical analysis: Chen Yuan; administrative, technical and material support: Yongning Xin; study supervision: Shiying Xuan.

Funding/Support: This study was supported by Qingdao livelihood, science and technology project, China (grant No.14-2-3-17-nsh) and Qingdao key health discipline development fund.

References

1. Clark JM. The epidemiology of nonalcoholic fatty liver disease in adults. *J Clin Gastroenterol.* 2006;**40 Suppl 1**:S5–10. doi: 10.1097/01.mcg.0000168638.84840.ff. [PubMed: 16540768]
2. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology.* 2005;**41**(6):1313–21. doi: 10.1002/hep.20701. [PubMed: 15915461]
3. Oh MK, Winn J, Poordad F. Review article: Diagnosis and treatment of non-alcoholic fatty liver disease. *Aliment Pharmacol Ther.* 2008;**28**(5):503–22. doi: 10.1111/j.1365-2036.2008.03752.x. [PubMed: 18532991]
4. Milic S, Stimac D. Nonalcoholic fatty liver disease/steatohepatitis: Epidemiology, pathogenesis, clinical presentation and treatment. *Dig Dis.* 2012;**30**(2):158–62. doi: 10.1159/000336669. [PubMed: 22722431]
5. Fan JG, Farrell GC. Epidemiology of non-alcoholic fatty liver disease in China. *J Hepatol.* 2009;**50**(1):204–10. doi: 10.1016/j.jhep.2008.10.010. [PubMed: 19014878]
6. Speliotes EK, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ, Palmer CD, et al. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. *PLoS Genet.* 2011;**7**(3):e1001324. doi: 10.1371/journal.pgen.1001324. [PubMed: 21423719]
7. Fischer M, Pleiss J. The Lipase Engineering Database: A navigation and analysis tool for protein families. *Nucleic Acids Res.* 2003;**31**(1):319–21. [PubMed: 12520012]
8. Lei X, Callaway M, Zhou H, Yang Y, Chen W. Obesity associated Lyplal1 gene is regulated in diet induced obesity but not required for adipocyte differentiation. *Mol Cell Endocrinol.* 2015;**411**:207–13. doi: 10.1016/j.mce.2015.05.001. [PubMed: 25958046]
9. Heid IM, Jackson AU, Randall JC, Winkler TW, Qi L, Steinthorsdottir V, et al. Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. *Nat Genet.* 2010;**42**(11):949–60. doi: 10.1038/ng.685. [PubMed: 20935629]
10. Jimenez MA, Akerblad P, Sigvardsson M, Rosen ED. Critical role for Ebf1 and Ebf2 in the adipogenic transcriptional cascade. *Mol Cell Biol.* 2007;**27**(2):743–57. doi: 10.1128/MCB.01557-06. [PubMed: 17060461]
11. Randall JC, Winkler TW, Kutalik Z, Berndt SI, Jackson AU, Monda KL, et al. Sex-stratified genome-wide association studies including 270,000 individuals show sexual dimorphism in genetic loci for anthropometric traits. *PLoS Genet.* 2013;**9**(6):e1003500. doi: 10.1371/journal.pgen.1003500. [PubMed: 23754948]
12. Fox CS, Liu Y, White CC, Feitosa M, Smith AV, Heard-Costa N, et al. Genome-wide association for abdominal subcutaneous and visceral adipose reveals a novel locus for visceral fat in women. *PLoS Genet.* 2012;**8**(5):e1002695. doi: 10.1371/journal.pgen.1002695. [PubMed: 22589738]
13. Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat Genet.* 2010;**42**(11):937–48. doi: 10.1038/ng.686. [PubMed: 20935630]
14. Rickham PP. Human Experimentation. Code of Ethics of the World Medical Association. Declaration of Helsinki. *Br Med J.* 1964;**2**(5402):177. [PubMed: 14150898]
15. Razavizade M, Jamali R, Arj A, Talari H. Serum parameters predict the severity of ultrasonographic findings in non-alcoholic fatty liver disease. *Hepatobiliary Pancreatic Dis Int.* 2012;**11**(5):513–20. doi: 10.1016/S1499-3872(12)60216-1.
16. Niu TH, Jiang M, Xin YN, Jiang XJ, Lin ZH, Xuan SY. Lack of association between apolipoprotein C3 gene polymorphisms and risk of nonalcoholic fatty liver disease in a Chinese Han population. *World J Gastroenterol.* 2014;**20**(13):3655–62. doi: 10.3748/wjg.v20.i13.3655. [PubMed: 24707151]
17. Kotronen A, Westerbacka J, Bergholm R, Pietilainen KH, Yki-Jarvinen H. Liver fat in the metabolic syndrome. *J Clin Endocrinol Metab.* 2007;**92**(9):3490–7. doi: 10.1210/jc.2007-0482. [PubMed: 17595248]
18. Kitamoto A, Kitamoto T, Nakamura T, Ogawa Y, Yoneda M, Hyogo H, et al. Association of polymorphisms in GCKR and TRIB1 with nonalcoholic fatty liver disease and metabolic syndrome traits. *Endocr J.* 2014;**61**(7):683–9. [PubMed: 24785259]
19. Lin YC, Chang PF, Chang MH, Ni YH. Genetic variants in GCKR and PNPLA3 confer susceptibility to nonalcoholic fatty liver disease in obese individuals. *Am J Clin Nutr.* 2014;**99**(4):869–74. doi: 10.3945/ajcn.113.079749. [PubMed: 24477042]
20. Palmer ND, Musani SK, Yerges-Armstrong LM, Feitosa MF, Bielak LF, Hernaez R, et al. Characterization of European ancestry nonalcoholic fatty liver disease-associated variants in individuals of African and Hispanic descent. *Hepatology.* 2013;**58**(3):966–75. doi: 10.1002/hep.26440. [PubMed: 23564467]
21. Gorden A, Yang R, Yerges-Armstrong LM, Ryan KA, Speliotes E, Borecki IB, et al. Genetic variation at NCAN locus is associated with inflammation and fibrosis in non-alcoholic fatty liver disease in morbid obesity. *Hum Hered.* 2013;**75**(1):34–43. doi: 10.1159/000346195. [PubMed: 23594525]
22. Petta S, Valenti L, Marchesini G, Di Marco V, Licata A, Camma C, et al. PNPLA3 GG genotype and carotid atherosclerosis in patients with non-alcoholic fatty liver disease. *PLoS One.* 2013;**8**(9):e74089. doi: 10.1371/journal.pone.0074089. [PubMed: 24069270]
23. Kawaguchi T, Sumida Y, Umemura A, Matsuo K, Takahashi M, Takamura T, et al. Genetic polymorphisms of the human PNPLA3 gene are strongly associated with severity of non-alcoholic fatty liver disease in Japanese. *PLoS One.* 2012;**7**(6):e38322. doi: 10.1371/journal.pone.0038322. [PubMed: 22427976]
24. Malhi H, Gores GJ. Molecular mechanisms of lipotoxicity in nonalcoholic fatty liver disease. *Semin Liver Dis.* 2008;**28**(4):360–9. doi: 10.1055/s-0028-1091980. [PubMed: 18956292]
25. Schindhelm RK, Diamant M, Dekker JM, Tushuizen ME, Teerlink T, Heine RJ. Alanine aminotransferase as a marker of non-alcoholic fatty liver disease in relation to type 2 diabetes mellitus and cardiovascular disease. *Diabetes Metab Res Rev.* 2006;**22**(6):437–43. doi: 10.1002/dmrr.666. [PubMed: 16832839]
26. Prati D, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, et al. Updated definitions of healthy ranges for serum alanine aminotransferase levels. *Ann Intern Med.* 2002;**137**(1):1–10. [PubMed: 12093239]

27. Pratt DS, Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. *N Engl J Med.* 2000;**342**(17):1266–71. doi: 10.1056/NEJM200004273421707. [PubMed: 10781624]
28. Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology.* 2003;**37**(4):917–23. doi: 10.1053/jhep.2003.50161. [PubMed: 12668987]
29. Leon-Mimila P, Vega-Badillo J, Gutierrez-Vidal R, Villamil-Ramirez H, Villareal-Molina T, Larrieta-Carrasco E, et al. A genetic risk score is associated with hepatic triglyceride content and nonalcoholic steatohepatitis in Mexicans with morbid obesity. *Exp Mol Pathol.* 2015;**98**(2):178–83. doi: 10.1016/j.yexmp.2015.01.012. [PubMed: 25597287]
30. Burke JE, Dennis EA. Phospholipase A2 biochemistry. *Cardiovasc Drugs Ther.* 2009;**23**(1):49–59. doi: 10.1007/s10557-008-6132-9. [PubMed: 18931897]
31. Burke JE, Dennis EA. Phospholipase A2 structure/function, mechanism, and signaling. *J Lipid Res.* 2009;**50 Suppl**:S237–42. doi: 10.1194/jlr.R800033-JLR200. [PubMed: 19011112]
32. He S, McPhaul C, Li JZ, Garuti R, Kinch L, Grishin NV, et al. A sequence variation (I148M) in PNPLA3 associated with nonalcoholic fatty liver disease disrupts triglyceride hydrolysis. *J Biol Chem.* 2010;**285**(9):6706–15. doi: 10.1074/jbc.M109.064501. [PubMed: 20034933]
33. Dixon JB, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. *Gastroenterology.* 2001;**121**(1):91–100. [PubMed: 11438497]