Association Between \textit{LYPLAL1} rs12137855 Polymorphism With Ultrasound-Defined Non-Alcoholic Fatty Liver Disease in a Chinese Han Population

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

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

\textbf{Patients and Methods:} \textit{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.

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

\textbf{Conclusions:} Our results showed for the first time that \textit{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.

\textbf{Keywords:} Single Nucleotide Polymorphism, Non-Alcoholic Fatty Liver Disease, \textit{LYPLAL1}
\end{abstract}

1. \textbf{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 \textit{LYPLAL1} rs12137855 for NAFLD in 7177 adults of European ancestry (6).

\textit{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 \textit{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 \textit{LYPLAL1} gene, which are associated with increased waist-hip ratio (WHR) adjusted for BMI only in women not men. SNP rs1118316 at \textit{LYPLAL1} is associated with visceral adipose tissue/subcutaneous adipose tissue ratio in both men and women (12), and SNP at rs12137855 near \textit{LYPLAL1} gene is strongly associated with NAFLD (13). Subsequent studies showed that \textit{LYPLAL1} plays an important role in fat distribution and lipid metabolism. \textit{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 to NAFLD patients (85 males, 97 females, mean age 43.18 ± 11.53 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’-TCCTAAGTTCCTATT-3’ and 5’-TGCTGTGGGGTGGTGAGTCA-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 χ² test. Genotype and alleles were estimated by chi-square test and DNA distributions between NAFLD patients and controls were analyzed by Pearson’s χ² 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 χ² 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 ($P_{\text{NAFLD}} = 0.323$; $P_{\text{control}} = 0.230$, 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
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| 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/m²      | 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 ± 8.63             | 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.

*Data are presented as mean ± SD.*

### Table 2. Association of Variants in *LYPLAL1* Gene with Risk of NAFLD

| SNP (rs12137855) | NAFLD Patients (n) | Controls (n) | $\chi^2$ | P Value |
|------------------|--------------------|--------------|---------|---------|
| Genotypes        |                    |              |         |         |
| CC               | 159                | 91           | 2.261   | .133    |
| CT               | 25                 | 23           |         |         |
| Alleles          |                    |              | 2.063   | .151    |
| C                | 343                | 205          |         |         |
| T                | 25                 | 23           |         |         |

Abbreviations: NAFLD, non-alcoholic fatty liver disease patients.

*P*: NAFLD patients vs. control.
Table 3. Clinical Characteristics of LYPLAL1 (rs12137855 C/C) Carriers and Non-Carriers in the Study Population4

| 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 | 167.35 ± 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 | 70.85 ± 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/m² | 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 ± 8.03 | 17.92 ± 7.93 | .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 ± 1.17 | 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.

4Data 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). For our study, we 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/m² or more is an index of obesity (16). In this study, BMI was higher in the NAFLD group and control group (25.34 ± 3.06 kg/m²) than controls (23.04 ± 3.47 kg/m²) (P < 0.05), also higher in the variant carrier (25.29 ± 3.51 kg/m²) than non-carrier (23.96 ± 3.82 kg/m²) 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 rs1237855 polymorphism and development of NAFLD in Chinese Han origin for the first time. The C allele of the rs1237855 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: Shiyong Xuan; statistical analysis: Chen Yuan; administrative, technical and material support: Yongning Xin; study supervision: Shiyong Xuan.

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