

# Patatin-like phospholipase domain-containing 3 gene (PNPLA3) polymorphic (rs738409) single nucleotide polymorphisms and susceptibility to nonalcoholic fatty liver disease A meta-analysis of twenty studies

Yan Zhao, MD, MM<sup>a</sup>, Wenli Zhao, MD, PhD<sup>b,c</sup>, Jingchao Ma, MPH<sup>d</sup>, Maeda Toshiyoshi, MM<sup>e</sup>, Ye Zhao, MD, PhD, MBA<sup>f,\*</sup>

#### Abstract

**Background:** To investigate the correlation between rs738409 polymorphism of patatin-like phospholipase domain-containing protein 3 (PNPLA3) gene (encoding I148m) and genetic susceptibility to nonalcoholic fatty liver disease (NAFLD).

**Methods:** Web of Science, Embase, PubMed, Cochrane Library, China National Knowledge Infrastructure, Wanfang Data Knowledge Service Platform databases were subjected to study retrieving, from the earliest records to November 2022. International databases were searched using the key words (PNPLA3 gene or PNPLA3 polymorphism or patatin-like phospholipase domain-containing pro-tein3) and (nonalcoholic fatty liver disease or NAFLD or nonalcoholic steatohepatitis) and their possible combination. There was no limitation to language. Ethnicity and country restrictions were not applied. Hardy–Weinberg equilibrium about the genotype frequencies of rs738,409 polymorphism in group of controls was assessed using a chi-square goodness-of-fit test (P > .05). A chi-square-based Q test was applied to assess heterogeneity among studies. The random-effect model (DerSimonian–Laird method) was used when a probability value of P < .10,  $l^2 > 50\%$ . If not, the fixed-effect model (Mantel–Haenszel method) was adopted. The current meta-analysis was done by using STATA 16.0.

**Results:** Twenty studies are selected for this meta-analysis, which includes totally 3240 patients in the treatment group and 5210 patients in the control group. These studies demonstrated a significant increased association between rs738,409 and NAFLD under 5 models: allelic contrast (odds ratio [OR] = 1.98, 95% confidence interval [CI] = 1.65-2.37,  $P_{heterogeneity} = 0.000$ , Z = 7.346, P = .000), homozygote comparison (OR = 3.59, 95% CI = 2.56-5.04,  $P_{heterogeneity} = 0.000$ , Z = 7.416, P = .000), heterozygote comparison (OR = 1.93, 95% CI = 1.63-2.30, *Pheterogeneity* = 0.002, Z = 7.507, P = .000), the dominant allele model (OR = 2.33, 95% CI = 1.89-2.88,  $P_{heterogeneity} = 0.000$ , Z = 7.856, P = .000), and the recessive allele model (OR = 2.56, 95% CI = 1.96-3.35,  $P_{heterogeneity} = 0.000$ , Z = 6.850, P = .000). Subgroup analysis shows that the rs738,409 polymorphism of PNPLA3 gene in Caucasians and those with a sample size of < 300 is significantly associated with the susceptibility to nonalcoholic fatty liver. Sensitivity analysis shows that the results of meta-analysis are stable.

Conclusion: PNPLA3 rs738,409 may play a significant role in increasing risk of NAFLD.

**Abbreviations:** NAFLD = nonalcoholic fatty liver disease, NASH = nonalcoholic steatohepatitis, OR = odds ratio, PNPLA3 = patatin-like phospholipase domain-containing protein 3, SNP = single nucleotide polymorphism, VLDL = very low-density lipoprotein.

Keywords: meta-analysis, NAFLD, PNPLA3, rs738409, SNP, susceptibility

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<sup>a</sup> Graduate school, Tianjin University of Traditional Chinese Medicine, Tianjin, China, <sup>b</sup> Department of Public Health, International College, Krirk University, Bangkok, Thailand, <sup>c</sup> Liver Center, Saga University Hospital, Saga University 849-8501, Saga City, Japan, <sup>d</sup> Department of Public Health, International College, Krirk University, Bangkok, Thailand, <sup>e</sup> International Education College, Shandong University of Traditional Chinese Medicine, Jinan, China, <sup>f</sup> Department of Public Health, International College, Krirk University, Bangkok, Thailand.

\* Correspondence: Ye Zhao, Department of Public Health, International College, Krirk University, Bangkok 10220, Thailand (e-mail: zhao.ye@staff.krirk.ac.th). Copyright © 2023 the Author(s). Published by Wolters Kluwer Health, Inc. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and buildup the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

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#### Figure 1. PRISMA 2009 flow diagram. PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

# 1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of liver disease.<sup>[1]</sup> It is a clinical syndrome characterized by diffuse hepatocyte steatosis, excluding excessive drinking and other clear liver damaging factors.<sup>[2]</sup> Due to changes in lifestyle, the prevalence of NAFLD increases at an alarming rate.<sup>[3]</sup> By 2018, the prevalence of NAFLD worldwide was 24.4%, including 20% to 30% in western countries and 5% to 18% in Asian countries.<sup>[4]</sup> Even in Africa, where the lowest prevalence is, the incidence rate is 14%.<sup>[5]</sup> The occurrence of NAFLD is mainly related to metabolic factors. It is mainly divided into 2 types: nonalcoholic fatty liver and nonalcoholic steatohepatitis (NASH). Nonalcoholic fatty liver is considered to be the beginning of the disease, which is caused by excessive accumulation of triglycerides in hepatocytes.<sup>[6]</sup> NASH can evolve into liver fibrosis, liver cirrhosis and finally into hepatocellular carcinoma. Hepatocellular carcinoma is the most serious consequence of NAFLD.<sup>[7]</sup> The pathogenesis of NAFLD is not clear.<sup>[8]</sup> Sensitivity of the liver to the damage increases insulin resistance.<sup>[9]</sup> It is an

important link and a risk factor in the progression of NAFLD. Insulin resistance leads to an increase of free fatty acids in serum. Subsequently, liver ingests a large amount of free fatty acids in the circulation for the lipid synthesis or the direct metabolism.<sup>[10]</sup> However, there are always some kinds of imbalance between lipid acquisition (lipid uptake and lipogenesis) and lipid clearance (lipid export and lipid oxidation). The continued accumulation of hepatic lipids will lead to lipid degeneration and even lipotoxicity.<sup>[11]</sup> Hepatocyte injury is caused by the secretion of inflammatory factors, oxidative stress, gut dysbiosis and mitochondrial dysfunction.<sup>[12]</sup> Furthermore, it deteriorates into NASH, liver fibrosis, cirrhosis.<sup>[13]</sup> No specific drug has been authorized for the treatment of NAFLD.<sup>[14]</sup> Health education and lifestyle interventions are therefore recommended as its first-line treatments.<sup>[15]</sup> Genetic factors cause NAFLD in 27% to 39% of cases. Different types of genes may involve in the pathogenesis of NAFLD. The key 1 is single nucleotide polymorphism (SNP) (rs738,409) in the patatin-like phospholipase domain-containing protein 3 (PNPLA3).

Table 1

| Characteristics of                    | the stud | ies included | in the meta-analy                           | sis.               |                 |                   |                   |
|---------------------------------------|----------|--------------|---|--------------------|-----------------|-------------------|-------------------|
| First author                          | Yr       | Country      | Age   | Gender (men/women) | NAFLD diagnosis | Source of control | Genotyping method |
| Hotta <sup>[25]</sup>                 | 2010     | Japan        | N:51.7±15.0<br>C:47.2+14.8                  | 304/527            | LB              | HB                | TaqMan            |
| Wang <sup>[26]</sup>                  | 2011     | China        | N:48.1 ± 12.95<br>C:45.4 ± 15.93            | 407/472            | US              | HB                | TaqMan            |
| Valenti <sup>[27]</sup>               | 2012     | Italy        | N:49.5±12<br>C:48+12                        | 314/87             | LB              | HB                | Real-time PCR     |
| Bhatt <sup>[28]</sup>                 | 2013     | India        | $N:38.2 \pm 7.0$<br>$C:37.1 \pm 6.9$        | NA                 | US              | HB                | Real-time PCR     |
| Rametta <sup>[29]</sup>               | 2014     | Italy        | N:49.7 $\pm$ 12.1<br>C:47.7 $\pm$ 12.1      | 314/83             | LB              | HB                | TaqMan            |
| Lee <sup>[30]</sup>                   | 2014     | Korea        | $N:44.1 \pm 15.5$<br>$C:45.3 \pm 10.6$      | 161/178            | US              | HB                | TaqMan            |
| Niu <sup>[31]</sup>                   | 2014     | China        | $N:49.76 \pm 16.17$<br>$C:47.69 \pm 15.86$  | 373/426            | US              | HB                | ABI sequencer     |
| Shang <sup>[32]</sup>                 | 2015     | China        | $N:11.81 \pm 2.20$<br>$C:11.44 \pm 2.99$    | 453/574            | US              | PB                | Mass ARRAY        |
| Vespasiani-Gentilucci <sup>[33]</sup> | 2016     | Italy        | $N:51.5 \pm 12.3$<br>C:40.1 + 13.1          | 84/101             | LB              | HB                | Sequencing        |
| Alam <sup>[34]</sup>                  | 2017     | Bangladesh   | $N:39.1 \pm 8.6$<br>$C:29.64 \pm 7.03$      | 75/99              | LB              | HB                | TaqMan            |
| Mazo <sup>[35]</sup>                  | 2018     | Brazil       | N:24-76<br>C:19-78                          | 127/255            | LB              | HB                | TaqMan            |
| Yang <sup>[36]</sup>                  | 2018     | China        | $N:70.95 \pm 4.73$<br>$C:72.53 \pm 5.67$    | 158/301            | US              | PB                | Real-time PCR     |
| Costanzo <sup>[37]</sup>              | 2019     | Italy        | $N:11.0 \pm 2.8$<br>C:9.6+2.7               | 131/99             | AMRI            | PB                | TaqMan            |
| Hudert <sup>[38]</sup>                | 2019     | Germany      | $N:14.11 \pm 2.15$<br>$C:46.73 \pm 16.03$   | 177/93             | LB              | PB                | TaqMan            |
| Wu <sup>[39]</sup>                    | 2020     | China        | $N:57.7 \pm 8.53$<br>$C:58.90 \pm 5.53$     | NA                 | US              | PB                | Real-time PCR     |
| Narayanasamy <sup>[40]</sup>          | 2020     | India        | $N:43.15 \pm 9.245$<br>$C:41.99 \pm 12.752$ | NA                 | US              | PB                | PCR-RFLP          |
| Lisboa <sup>[41]</sup>                | 2020     | Brazil       | N:46.3 - 63<br>C:43 - 61 5                  | 77/208             | LB              | HB                | Real-time PCR     |
| Delik <sup>[42]</sup>                 | 2020     | Turkey       | $N:46.09 \pm 10$<br>C:44.69 + 11.28         | 155/174            | LB              | HB                | Real-time TaqMan  |
| Akkiz <sup>[43]</sup>                 | 2021     | Turkey       | $N:47.04 \pm 12.2$<br>$C:26.9 \pm 8.6$      | NA                 | LB              | HB                | Real-time PCR     |
| Zhang <sup>[44]</sup>                 | 2021     | China        | N:38.2±13.78<br>C:42.2±11.05                | 102/50             | LB              | HB                | Real-time PCR     |

AMRI = abdominal magnetic resonance imaging, HB = hospital-based study, LB = liver biopsy, NA = Not available, NAFLD = nonalcoholic fatty liver disease, PB = population based, PCR = polymerase chain reaction-restriction fragment length polymorphisms, US = liver ultrasonographic examination.

PNPLA3 is also known to be a lipoatrophic protein consisting of 481 amino acids and belongs to the PNPLA family.<sup>[16]</sup> PNPLA3 is a transmembrane protein which is mainly expressed in hepatocytes. It is also expressed in adipocytes and skin, which is regulated by the nutritional status. PNPLA3 influences the hepatic fat metabolism importantly.<sup>[17]</sup> It confirmed that PNPLA3 possesses triacylglycerol hydrolase and acylglycerol transacylase activities.<sup>[18]</sup> Its gene mutation may affect fat metabolism, including the fat synthesis and the fat hydrolysis.<sup>[19]</sup> The PNPLA3 gene is located on human chromosome 22. The most studied focus on PNPLA3 gene, rs738,409 [g/ C], encodes i148m.<sup>[20]</sup> The i148m mutation is assigned isoleucine (I) at position 148 and methionine (M) at position 148, which causes a guanine to cytosine substitution ( $\hat{C}\rightarrow G$ ). It impairs the function of the protein and perturbs the intracellular triglyceride metabolism. Then triglyceride accumulation is increased in hepatocytes.[21] The first genome-wide association study was performed in 2008 in a cohort of Hispanics, African Americans, and European Americans. It is found that PNPLA3 rs738,409 polymorphism is associated with the increased liver fat content and liver inflammation level.<sup>[22]</sup> The G allele of PNPLA3 rs738,409 is found to increase the risk of steatosis, lobular inflammation, hepatocyte ballooning.<sup>[23]</sup> This anatomically confirms its association with NAFLD development and progression. There was no association with body mass index, triglyceride levels, high density lipoprotein and low-density lipoprotein, or diabetes. It is not found the relationship between rs738,409 with NASH or hepatocyte ballooning.<sup>[24]</sup> Therefore, the aim of this paper is to conduct a meta-analysis of relevant studies. It will demonstrate that the PNPLA3 rs738,409 polymorphism may impact on the NAFLD susceptibility.

#### 2. Methods

#### 2.1. Article search strategy

Web of Science, Embase, PubMed, Cochrane Library, China National Knowledge Infrastructure, Wanfang Data Knowledge Service Platform databases were subjected to study retrieving, from the earliest records to November 2022. International databases were searched using the key words (PNPLA3 gene or PNPLA3 polymorphism or patatin-like phospholipase domain-containing pro-tein3) and (nonalcoholic fatty liver disease or NAFLD or nonalcoholic steatohepatitis) and their possible combination. There was no limitation to language. Ethnicity and country restrictions were not applied.

|                       | Sar   | nple size | Gen | otype in | cases | Geno | type in ( | controls | C   | ase | Co   | ntrol |                |                 |
|-----------------------|-------|-----------|-----|----------|-------|------|-----------|----------|-----|-----|------|-------|----------------|-----------------|
| First author          | Cases | Controls  | CC  | CG       | GG    | CC   | CG        | GG       | C   | G   | C    | G     | HWE (controls) | PH-W (controls) |
| Hotta                 | 253   | 578       | 45  | 111      | 97    | 175  | 296       | 104      | 201 | 305 | 646  | 504   | Yes            | 0.28            |
| Wang                  | 156   | 723       | 40  | 80       | 36    | 269  | 335       | 119      | 160 | 152 | 873  | 573   | Yes            | 0.42            |
| Valenti               | 144   | 257       | 55  | 68       | 21    | 146  | 95        | 16       | 178 | 110 | 387  | 127   | Yes            | 0.87            |
| Bhatt                 | 162   | 173       | 112 | 35       | 15    | 149  | 16        | 8        | 259 | 65  | 314  | 32    | No             | 0.0000          |
| Rametta               | 137   | 260       | 51  | 67       | 19    | 150  | 95        | 15       | 169 | 105 | 395  | 125   | Yes            | 0.96            |
| Lee                   | 155   | 184       | 31  | 75       | 49    | 55   | 92        | 37       | 137 | 173 | 202  | 166   | Yes            | 0.97            |
| Niu                   | 390   | 409       | 48  | 153      | 189   | 183  | 176       | 50       | 249 | 531 | 542  | 276   | Yes            | 0.33            |
| Shang                 | 162   | 865       | 60  | 74       | 28    | 338  | 418       | 109      | 194 | 130 | 1094 | 636   | Yes            | 0.22            |
| Vespasiani-Gentilucci | 60    | 125       | 29  | 18       | 13    | 83   | 34        | 8        | 76  | 44  | 200  | 50    | Yes            | 0.08            |
| Alam                  | 99    | 75        | 37  | 43       | 19    | 45   | 27        | 3        | 117 | 81  | 117  | 33    | Yes            | 0.62            |
| Mazo                  | 248   | 134       | 77  | 117      | 54    | 66   | 55        | 13       | 271 | 225 | 187  | 81    | Yes            | 0.75            |
| Yang                  | 97    | 362       | 27  | 40       | 30    | 110  | 123       | 129      | 94  | 100 | 343  | 381   | No             | 0.0000          |
| Costanzo              | 105   | 125       | 32  | 55       | 18    | 76   | 45        | 4        | 119 | 91  | 197  | 98    | No             | 0.000           |
| Hudert                | 70    | 200       | 20  | 31       | 19    | 118  | 71        | 11       | 71  | 69  | 307  | 93    | Yes            | 0.74            |
| Wu                    | 242   | 266       | 73  | 121      | 48    | 106  | 128       | 32       | 267 | 217 | 340  | 192   | No             | 0.46            |
| Naravanasamv          | 105   | 102       | 19  | 50       | 36    | 59   | 29        | 14       | 88  | 122 | 147  | 57    | No             | 0.004           |
| Lisboa                | 148   | 137       | 55  | 65       | 28    | 80   | 43        | 14       | 175 | 121 | 203  | 71    | No             | 0.03            |
| Delik                 | 248   | 81        | 76  | 83       | 89    | 42   | 22        | 17       | 235 | 261 | 106  | 56    | No             | 0.0002          |
| Akkiz                 | 200   | 61        | 57  | 63       | 80    | 33   | 15        | 13       | 177 | 223 | 81   | 41    | No             | 0.0005          |
| Zhang                 | 59    | 93        | 12  | 27       | 20    | 35   | 43        | 15       | 51  | 67  | 113  | 73    | Yes            | 0.68            |

HWE = Hardy-Weinberg equilibrium, NAFLD = nonalcoholic fatty liver disease, PNPLA3 = patatin-like phospholipase domain-containing protein 3.

# Table 3

Quality of literature included in the study.

| study                             | Adequate<br>definition<br>of cases | Representativeness of the cases | Selection<br>of<br>controls | Definition<br>of<br>controls | Comparability of cases<br>and controls on the basis<br>of the design or analysis | Ascertainment<br>of exposure | Same method of<br>ascertainment for<br>cases and controls | Nonresponse<br>rate | Quality<br>score |
|-----------------------------------|------------------------------------|---------------------------------|-----------------------------|------------------------------|--|------------------------------|---|---------------------|------------------|
| Hotta 2010                        | ☆                                  | \$                              | ☆                           | ☆                            | **   | ☆                            | \$  |                     | 8                |
| Wang 2011                         | ☆                                  | ☆                               |                             | ☆                            | **   |                              | ☆   | ☆                   | 7                |
| Valenti 2012                      | ☆                                  | ☆                               | ☆                           | ☆                            | \$   | ☆                            | \$  | ☆                   | 8                |
| Bhatt 2013                        | ☆                                  | ☆                               | ☆                           |                              | **   | ☆                            | ☆   | ☆                   | 8                |
| Rametta 2014                      | ☆                                  | ☆                               |                             | ☆                            | \$   | ☆                            | \$  | ☆                   | 7                |
| Lee 2014                          | ☆                                  | ☆                               | ☆                           |                              | \$   | ☆                            |   | ☆                   | 6                |
| Niu 2014                          | ☆                                  | ☆                               |                             | ☆                            | ☆  | ☆                            | ☆   | ☆                   | 7                |
| Shang 2015                        | ☆                                  | ☆                               | ☆                           | ☆                            | \$   | ☆                            | \$  | \$                  | 8                |
| Vespasiani-<br>Gentilucci<br>2016 | ☆                                  | ☆                               | *                           |                              | **   | ☆                            | *   |                     | 7                |
| Alam 2017                         | ☆                                  | ☆                               |                             | ☆                            | ☆  | ☆                            | *   | ☆                   | 7                |
| Mazo 2018                         | ☆                                  | \$                              |                             | \$                           | \$   | \$                           | \$  | \$                  | 7                |
| Yang 2018                         | ☆                                  | \$                              | ☆                           | \$                           | *  | \$                           | \$  | \$                  | 8                |
| Costanzo<br>2019                  | \$                                 | \$                              |                             | \$                           | **   | ☆                            | \$  |                     | 7                |
| Hudert 2019                       | ☆                                  | ☆                               | ☆                           | ☆                            | **   | ☆                            | ☆   | ☆                   | 9                |
| Wu 2020                           | ☆                                  | ☆                               |                             | ☆                            | **   | ☆                            | ☆   | ☆                   | 8                |
| Narayanasamy<br>2020              | \$                                 | \$                              | \$                          |                              | **   | \$                           | ☆   |                     | 7                |
| Lisboa 2020                       | ☆                                  | ☆                               | ☆                           |                              | **   | ☆                            | ☆   | ☆                   | 8                |
| Delik 2020                        | ☆                                  | \$                              | ☆                           |                              | **   | ☆                            | ☆   |                     | 7                |
| Akkiz 2021                        | ☆                                  | \$                              |                             | ☆                            | **   | ☆                            | ☆   |                     | 7                |
| Zhang 2021                        | \$                                 | \$                              | \$                          |                              | $\Delta \Delta$  | ☆                            | ☆   | \$                  | 8                |

## 2.2. Inclusive criteria

All the eligible studies were selected on the basis of the predefined inclusion criteria:

- 1) evaluation of association between NAFLD and rs738,409 G/C polymorphism;
- 2) case-control studies based on populations or hospitals;
- 3) studies with original data;
- 4) studies with a clear diagnosis of NAFLD;
- 5) the allele frequency provided by articles ought to be sufficient for calculating genotypic odds ratio (OR) combined

## 2.3. Exclusion criteria

cases and controls.

Articles are excluded if they met any 1 of the following criteria:

with corresponding 95% confidence interval (CI) in both

- 1) repeated records;
- 2) review articles, editorial comments, case reports and animal studies;
- 3) no information on genotype frequency;

Α

В

|                              |          | Odds Ratio        | %      |
|------------------------------|----------|-------------------|--------|
| study (year)                 |          | (95% CI)          | Weight |
| Hotta (2010)                 | -*-      | 1.94 (1.57, 2.41) | 5.66   |
| Wang (2011)                  |          | 1.45 (1.13, 1.85) | 5.52   |
| Valenti (2012)               |          | 1.88 (1.38, 2.57) | 5.20   |
| Bhatt (2013)                 |          | 2.46 (1.56, 3.88) | 4.44   |
| Rametta (2014)               |          | 1.96 (1.43, 2.69) | 5.18   |
| Lee (2014)                   |          | 1.54 (1.13, 2.08) | 5.24   |
| Niu (2014)                   |          | 4.19 (3.40, 5.16) | 5.67   |
| Shang (2015)                 |          | 1.15 (0.90, 1.47) | 5.53   |
| Vespasiani-Gentilucci (2016) | <b>*</b> | 2.32 (1.43, 3.76) | 4.28   |
| Alam (2017)                  |          | 2.45 (1.52, 3.96) | 4.31   |
| Mazo (2018)                  |          | 1.92 (1.40, 2.63) | 5.18   |
| Yang (2018)                  |          | 0.96 (0.70, 1.32) | 5.17   |
| Costanzo (2019)              |          | 1.54 (1.07, 2.21) | 4.92   |
| Hudert (2019)                |          | 3.21 (2.14, 4.81) | 4.71   |
| Wu (2020)                    |          | 1.44 (1.12, 1.85) | 5.49   |
| Narayanasamy (2020)          |          | 3.58 (2.37, 5.39) | 4.67   |
| Lisboa (2020)                |          | 1.98 (1.38, 2.82) | 4.97   |
| Delik (2020)                 |          | 2.10 (1.45, 3.04) | 4.90   |
| Akkiz (2021)                 |          | 2.49 (1.63, 3.80) | 4.60   |
| Zhang (2021)                 |          | 2.03 (1.27, 3.25) | 4.36   |
|                              |          | 1 98 (1 65 2 37)  | 100.00 |

# allelic model (G vs C)

| study (year)                                    | Odds Ratio<br>(95% CI) | %<br>Weight |
|---|------------------------|-------------|
| Hotta (2010)                                    | 3.63 (2.36, 5.57)      | 5.95        |
| Wang (2011) —                                   | 2.03 (1.23, 3.35)      | 5.75        |
| Valenti (2012)                                  | 3.48 (1.69, 7.16)      | 5.06        |
| Bhatt (2013)                                    | 2.49 (1.02, 6.09)      | 4.51        |
| Rametta (2014)                                  | 3.73 (1.76, 7.87)      | 4.97        |
| Lee (2014)                                      | 2.35 (1.27, 4.34)      | 5.40        |
| Niu (2014)                                      | 14.41 (9.23, 22.49)    | 5.90        |
| Shang (2015)                                    | 1.45 (0.88, 2.38)      | 5.75        |
| Vespasiani-Gentilucci (2016)                    | 4.65 (1.75, 12.35)     | 4.24        |
| Alam (2017)                                     | 7.70 (2.11, 28.07)     | 3.35        |
| Mazo (2018)                                     | 3.56 (1.79, 7.09)      | 5.16        |
| Yang (2018)                                     | 0.95 (0.53, 1.69)      | 5.51        |
| Costanzo (2019)                                 | 10.69 (3.35, 34.07)    | 3.71        |
| Hudert (2019)                                   | 10.19 (4.22, 24.59)    | 4.54        |
| Wu (2020) —                                     | 2.18 (1.27, 3.73)      | 5.64        |
| Narayanasamy (2020)                             | 7.98 (3.57, 17.86)     | 4.79        |
| Lisboa (2020) -                                 | 2.91 (1.41, 6.02)      | 5.04        |
| Delik (2020) -                                  | 2.89 (1.52, 5.49)      | 5.32        |
| Akkiz (2021)                                    | 3.56 (1.72, 7.36)      | 5.04        |
| Zhang (2021) -                                  | 3.89 (1.52, 9.93)      | 4.37        |
| Overall, DL (l <sup>2</sup> = 80.1%, p = 0.000) | 3.59 (2.56, 5.04)      | 100.00      |
|   | 1                      |             |

## homozygote model (GG vs CC)

Figure 2. Gene Models.

- 4) articles with unclearly described original data;
- 5) studies without controls groups;
- 6) studies with secondary causes of steatosis, including alcohol abuse, the use of drugs, surgical procedures and hepatitis B and hepatitis C virus infection.

# 2.4. Data extraction and quality evaluation

Two reviewers independently assessed and reviewed all identified studies in terms of inclusion and exclusion

criteria. Conflicts were reached to agreement via the discussion with the third authors. We extracted the following elements from each qualified study: the first author, the publication year, the country of origin, the sex ratio, the mean age, diagnostic criteria for NAFLD, the genetic detection method, the source of control group, the number of individuals in 2 groups, the frequency of PNPLA3 genotypes in 2 groups; the Hardy–Weinberg equilibrium test in control group and the *P* value of Hardy–Weinberg equilibrium. We used the Newcastle–Ottawa Scale to evaluate the E

|   | Odds Ratio          | %      |
|---|---------------------|--------|
| study (year)                                    | (95% CI)            | Weigh  |
| Hotta (2010)                                    | 2.02 (1.40, 2.92)   | 5.73   |
| Wang (2011) -                                   | 1.72 (1.16, 2.54)   | 5.61   |
| Valenti (2012)                                  | 2.13 (1.40, 3.23)   | 5.46   |
| Bhatt (2013)                                    | 2.77 (1.61, 4.78)   | 4.74   |
| Rametta (2014)                                  | 2.30 (1.50, 3.52)   | 5.41   |
| Lee (2014)                                      | ■ 1.71 (1.03, 2.82) | 4.97   |
| Niu (2014)                                      | 5.77 (4.03, 8.27)   | 5.77   |
| Shang (2015)                                    | 1.09 (0.77, 1.54)   | 5.84   |
| Vespasiani-Gentilucci (2016)                    | 2.11 (1.13, 3.96)   | 4.30   |
| Alam (2017)                                     | 2.51 (1.36, 4.65)   | 4.36   |
| Mazo (2018)                                     | 2.16 (1.40, 3.32)   | 5.37   |
| Yang (2018)                                     | 1.13 (0.69, 1.86)   | 5.01   |
| Costanzo (2019)                                 | 3.54 (2.04, 6.13)   | 4.72   |
| Hudert (2019)                                   | 3.60 (1.99, 6.49)   | 4.50   |
| Wu (2020)                                       | 1.53 (1.06, 2.22)   | 5.73   |
| Narayanasamy (2020)                             | 6.21 (3.30, 11.70)  | 4.27   |
| Lisboa (2020)                                   | 2.37 (1.47, 3.82)   | 5.13   |
| Delik (2020)                                    | 2.44 (1.46, 4.07)   | 4.92   |
| Akkiz (2021)                                    | 2.96 (1.64, 5.33)   | 4.50   |
| Zhang (2021)                                    | 2.36 (1.11, 5.05)   | 3.65   |
| Overall, DL (l <sup>2</sup> = 74.5%, p = 0.000) | 2.33 (1.89, 2.88)   | 100.00 |
|   | 1                   |        |
| .0625 1   | 16                  |        |

dominant allele model (CG+GG VS CC)

Figure 2. Continued

quality of the literatures included in the study. The evaluation content mainly includes 3 aspects: Selection (Adequate definition of cases, representativeness of the cases, selection of controls, and definition of controls); Comparability (Comparability of cases and controls on the basis of the design or analysis); and Exposure (Ascertainment of exposure, same method of ascertainment for cases and controls, and nonresponse rate). According to the above items, asterisks are marked on each document. And the asterisks' quantity has a positive correlation with the quality of documents.

#### 2.5. Statistical analysis

Hardy-Weinberg equilibrium about the genotype frequencies of rs738,409 polymorphism in group of controls is assessed using a chi-square goodness-of-fit test(P > .05). The combination of ORs and the corresponding 95% CIs is computed to estimate the correlation between rs738,409 polymorphism and NAFLD risk. For quantitative variables, standardized mean difference and corresponding 95% CI are used as a measure of effect size. A chi-square-based Q test is applied to assess heterogeneity among studies. The random-effect model (DerSimonian-Laird method) is used when a probability value of P < .10,  $I^2 > 50\%$ . If not, the fixed-effect model (Mantel-Haenszel method) is adopted. Generally, the pooled effect of correlation between rs738409 polymorphism and NAFLD susceptibility is assessed in 5 genetic models: allele model (G vs C), homozygote (co-dominant) model (GG vs CC), heterozygote (co-dominant) model (CG vs CC), dominant model (CG + GG vs CC) and recessive model (GG vs CC + CG). The stratified analyses according to ethnicity (Asian and Caucasian), total sample size (< 300 or more than 300 in case groups and control groups) is further conducted to recognize the differences among subgroups. Publication bias are evaluated by the funnel plot asymmetry, which uses the Begg test. P values < .05 are considered to be significant. The current meta-analysis

is analyzed by STATA 16.0 (Stata Corporation, College Station, TX).

#### 3. Results

### 3.1. Literature search

The flow diagram of study selection is presented in Figure 1. The search strategy initially identified 4171 potentially relevant articles, after the full-text reading, the repeated publications with the same data and review articles were excluded, leaving 2570 articles. After another round of full-text reading, based on the inclusion and exclusion criteria, and in total 20 articles are finally included in this study.

#### 3.2. Basic information

Basic information of the 20 included articles is shown in Table 1. Seven articles focus on the Caucasian population. Thirteen articles focus on the Asian population, including 8450 subjects from 9 countries or regions. There were 3240 patients with NAFLD as case group and 5210 subjects with no NAFLD as control group. Diagnostic criteria for NAFLD include the liver biopsy, the liver ultrasonographic examination, and the abdominal magnetic resonance imaging. Gene detection methods include the first generation of a direct sequencing technique, the TaqMan probe technique, the polymerase chain reaction-restriction fragment length polymorphisms, and the Matrix assisted laser desorption/ionization time of flight mass spectrometry. Moreover, there are 10 case-control articles based on populations and 10 case-control articles based on hospitals. In addition, 12 studies in the genotype distribution of the control group are consistent with the Hardy-Weinberg equilibrium. The genotype distribution of the 20 articles is described in detail in Table 2. The quality evaluation of the literature included in the study is shown in Table 3. There is no missing data in the collection or collation of literatures.

С

|            |        | (95% Cl)<br>1.46 (0.98, 2.16)<br>1.61 (1.06, 2.42)<br>1.90 (1.22, 2.95)<br>2.91 (1.53, 5.52) | Weight<br>6.38<br>6.18<br>5.89  |
|------------|--------|--|---|
|            |        | 1.46 (0.98, 2.16)<br>1.61 (1.06, 2.42)<br>1.90 (1.22, 2.95)<br>2.91 (1.53, 5.52)             | 6.38<br>6.18<br>5.89  |
|            | -<br>- | 1.61 (1.06, 2.42)<br>1.90 (1.22, 2.95)<br>2.91 (1.53, 5.52)                                  | 6.18<br>5.89  |
|            |        | 1.90 (1.22, 2.95)<br>2.91 (1.53, 5.52)   | 5.89  |
|            | -      | 2.91 (1.53, 5.52)  |   |
| +*-        | -      |  | 4.12  |
| +          |        | 2.07 (1.33, 3.24)  | 5.83  |
|            |        | 1.45 (0.85, 2.47)  | 4.97  |
|            | •      | 3.31 (2.26, 4.87)  | 6.4   |
| -          |        | 1.00 (0.69, 1.44)  | 6.64  |
| -          | -      | 1.52 (0.74, 3.08)  | 3.64  |
| -          | _      | 1.94 (1.01, 3.71)  | 4.0   |
|            |        | 1.82 (1.15, 2.89)  | 5.6   |
|            |        | 1.32 (0.76, 2.30)  | 4.8   |
|            |        | 2.90 (1.64, 5.14)  | 4.6   |
|            |        | 2.58 (1.37, 4.86)  | 4.16  |
| - <u>-</u> |        | 1.37 (0.93, 2.02)  | 6.4   |
|            | •      | - 5.35 (2.68, 10.68)   | 3.77  |
|            | _      | 2.20 (1.31, 3.68)  | 5.1   |
|            | _      | 2.08 (1.14, 3.81)  | 4.4   |
|            |        | 2.43 (1.20, 4.93)  | 3.6   |
| -          |        | 1.83 (0.81, 4.13)  | 3.0   |
|            |        | 1.93 (1.63, 2.30)  | 100.00  |
| 1          |        |  |   |
|            |        |  | 1.45 (0.65, 2.47)<br>3.31 (2.26, 4.87)<br>1.00 (0.69, 1.44)<br>1.52 (0.74, 3.08)<br>1.52 (0.74, 3.08)<br>1.32 (0.76, 2.30)<br>2.90 (1.64, 5.14)<br>2.58 (1.37, 4.86)<br>1.37 (0.93, 2.02)<br>5.35 (2.68, 10.68)<br>2.20 (1.31, 3.88)<br>2.08 (1.14, 3.81)<br>2.43 (1.20, 4.93)<br>1.83 (0.81, 4.13)<br>1.93 (1.63, 2.30)<br>1.93 (1.63, 2.30) |

heterozygote model (CG vs CC)



recessive allele model (GG vs CC+CG)

Figure 2. Continued

#### 3.3. Meta-analysis results

These studies demonstrate a significant increasing association between rs738,409 and NAFLD under 5 models: the allelic contrast (OR = 1.98, 95% CI = 1.65–2.37,  $P_{\text{heterogeneity}} = 0.000$ , Z = 7.346, P = .000), the homozygote comparison (OR = 3.59, 95% CI = 2.56–5.04,  $P_{\text{heterogeneity}} = 0.000$ , Z = 7.416, P = .000), the heterozygote comparison (OR = 1.93, 95% CI = 1.63–2.30,  $P_{\text{heterogeneity}} = 0.002$ , Z = 7.507, P = .000), the dominant allele model (OR = 2.33, 95% CI = 1.89–2.88,  $P_{\text{heterogeneity}} = 0.000$ , Z

= 7.856, P = .000), and the recessive allele model (OR = 2.56, 95% CI = 1.96–3.35,  $P_{\text{heterogeneity}} = 0.000$ , Z = 6.850, P = .000) (Fig. 2). In order to find potential sources of heterogeneity, we further conducted the subgroup analysis. For races and sample sizes of the 5 studies, it shows that the heterogeneity is significantly reduced in Caucasians. There is a significant correlation between rs738,409 and NAFLD susceptibility. Similarly, when the sample size is < 300, the heterogeneity is small, and the correlation is strong (Fig. 3). Among them, 4 articles mentioned

Α

| country and study (year)                         | Odds Ratio<br>(95% CI) | %<br>Weight |
|--|------------------------|-------------|
| Asian  |                        |             |
| Hotta (2010)                                     | 1.94 (1.57, 2.41)      | 5.66        |
| Wang (2011)                                      |                        | 5.52        |
| Bhatt (2013)                                     | 2.46 (1.56, 3.88)      | 4.44        |
| Lee (2014)                                       | 1.54 (1.13, 2.08)      | 5.24        |
| Niu (2014)                                       | 4.19 (3.40, 5.16)      | 5.67        |
| Shang (2015)                                     | 1.15 (0.90, 1.47)      | 5.53        |
| Alam (2017)                                      | 2.45 (1.52, 3.96)      | 4.31        |
| Yang (2018)                                      | 0.96 (0.70, 1.32)      | 5.17        |
| Wu (2020)  | 1.44 (1.12, 1.85)      | 5.49        |
| Narayanasamy (2020)                              | 3.58 (2.37, 5.39)      | 4.67        |
| Delik (2020)                                     | 2.10 (1.45, 3.04)      | 4.90        |
| Akkiz (2021)                                     | 2.49 (1.63, 3.80)      | 4.60        |
| Zhang (2021)                                     | 2.03 (1.27, 3.25)      | 4.36        |
| Subgroup, DL (I <sup>2</sup> = 89.5%, p = 0.000) | 1.95 (1.50, 2.53)      | 65.56       |
| Caucasian  |                        |             |
| Valenti (2012)                                   | 1.88 (1.38, 2.57)      | 5.20        |
| Rametta (2014)                                   | 1.96 (1.43, 2.69)      | 5.18        |
| Vespasiani-Gentilucci (2016)                     | 2.32 (1.43, 3.76)      | 4.28        |
| Mazo (2018)                                      | 1.92 (1.40, 2.63)      | 5.18        |
| Costanzo (2019)                                  | 1.54 (1.07, 2.21)      | 4.92        |
| Hudert (2019)                                    | 3.21 (2.14, 4.81)      | 4.71        |
| Lisboa (2020)                                    | 1.98 (1.38, 2.82)      | 4.97        |
| Subgroup, DL (I <sup>2</sup> = 23.3%, p = 0.251) | 2.02 (1.73, 2.35)      | 34.44       |
| Heterogeneity between groups: p = 0.818          |                        |             |
| Overall, DL (1 <sup>2</sup> = 84.4%, p = 0.000)  | 1.98 (1.65, 2.37)      | 100.00      |

NOTE: Weights and between-subgroup heterogeneity test are from random-effects model

| count and study (year)                           | Odds Ratio<br>(95% CI) | %<br>Weight |
|--|------------------------|-------------|
| >300   |                        |             |
| Hotta (2010) -                                   | 1.94 (1.57, 2.41)      | 5.66        |
| Wang (2011)                                      | 1.45 (1.13, 1.85)      | 5.52        |
| Valenti (2012)                                   | 1.88 (1.38, 2.57)      | 5.20        |
| Bhatt (2013) -                                   | 2.46 (1.56, 3.88)      | 4.44        |
| Rametta (2014)                                   | 1.96 (1.43, 2.69)      | 5.18        |
| Lee (2014)                                       | 1.54 (1.13, 2.08)      | 5.24        |
| Niu (2014)                                       | 4.19 (3.40, 5.16)      | 5.67        |
| Shang (2015)                                     | 1.15 (0.90, 1.47)      | 5.53        |
| Mazo (2018)                                      | 1.92 (1.40, 2.63)      | 5.18        |
| Yang (2018)                                      | 0.96 (0.70, 1.32)      | 5.17        |
| Wu (2020)  | 1.44 (1.12, 1.85)      | 5.49        |
| Delik (2020)                                     | 2.10 (1.45, 3.04)      | 4.90        |
| Subgroup, DL (l <sup>2</sup> = 89.1%, p = 0.000) | 1.78 (1.39, 2.28)      | 63.18       |
| <300   |                        |             |
| Vespasiani-Gentilucci (2016)                     | 2.32 (1.43, 3.76)      | 4.28        |
| Alam (2017) -                                    | 2.45 (1.52, 3.96)      | 4.31        |
| Costanzo (2019)                                  | 1.54 (1.07, 2.21)      | 4.92        |
| Hudert (2019)                                    | 3.21 (2.14, 4.81)      | 4.71        |
| Narayanasamy (2020)                              | 3.58 (2.37, 5.39)      | 4.67        |
| Lisboa (2020)                                    | 1.98 (1.38, 2.82)      | 4.97        |
| Akkiz (2021) -                                   | 2.49 (1.63, 3.80)      | 4.60        |
| Zhang (2021)                                     | 2.03 (1.27, 3.25)      | 4.36        |
| Subgroup, DL (l <sup>2</sup> = 45.5%, p = 0.076) | 2.35 (1.93, 2.88)      | 36.82       |
| Heterogeneity between groups: p = 0.086          |                        |             |
| Overall, DL (l <sup>2</sup> = 84.4%, p = 0.000)  | 1.98 (1.65, 2.37)      | 100.00      |
| 25 1   | 4                      |             |
|  | 4                      |             |

allelic model (G vs C)

Figure 3. Subgroup analysis.

alanine aminotransferase and 4 articles mentioned aspartate aminotransferase. As shown in Figure 4, there is a great heterogeneity. However, there is no statistical difference.

**3.3.1. Sensitivity study.** A sensitivity study was performed to estimate the effects of each individual study on the pooled OR. The sensitivity analysis indicates that none of the individual study alters the pooled results significantly. It confirms that our results are credible and generalizable (Fig. 5).

**3.3.2.** Publication bias. The Begg funnel plot was conducted to evaluate publication bias. As the dominant model shown in

Figure 6, the shape of the funnel plot shows good symmetry. It shows that the publication bias is small.

## 4. Discussion

PNPLA3-rs738,409 gene polymorphisms and NAFLD susceptibility are controversial. It reports that there is no correlation between PNPLA3-rs738,409 gene polymorphism and hepatic steatosis.<sup>[45]</sup> However, some other research shows that they are closely related.<sup>[46]</sup> Our study focuses on the relationship between PNPLA3 rs738,409 gene diversity and NAFLD. Ε

| country and study (year)                         | (95% CI)            | %<br>Weight |
|--|---------------------|-------------|
| Asian  |                     |             |
| Hotta (2010)                                     | - 2.02 (1.40, 2.92) | 5.73        |
| Wang (2011)                                      | 1.72 (1.16, 2.54)   | 5.61        |
| Bhatt (2013)                                     | 2.77 (1.61, 4.78)   | 4.74        |
| Lee (2014)                                       | - 1.71 (1.03, 2.82) | 4.97        |
| Niu (2014)                                       | 5.77 (4.03, 8.27)   | 5.77        |
| Shang (2015)                                     | 1.09 (0.77, 1.54)   | 5.84        |
| Alam (2017)                                      | 2.51 (1.36, 4.65)   | 4.36        |
| Yang (2018)                                      | 1.13 (0.69, 1.86)   | 5.01        |
| Wu (2020)  | 1.53 (1.06, 2.22)   | 5.73        |
| Narayanasamy (2020)                              | 6.21 (3.30, 11.70)  | 4.27        |
| Delik (2020)                                     | 2.44 (1.46, 4.07)   | 4.92        |
| Akkiz (2021)                                     | 2.96 (1.64, 5.33)   | 4.50        |
| Zhang (2021)                                     | 2.36 (1.11, 5.05)   | 3.65        |
| Subgroup, DL (I <sup>2</sup> = 82.5%, p = 0.000) | > 2.25 (1.65, 3.07) | 65.11       |
| Caucasian  |                     |             |
| Valenti (2012)                                   | 2.13 (1.40, 3.23)   | 5.46        |
| Rametta (2014)                                   | 2.30 (1.50, 3.52)   | 5.41        |
| Vespasiani-Gentilucci (2016)                     | 2.11 (1.13, 3.96)   | 4.30        |
| Mazo (2018)                                      | 2.16 (1.40, 3.32)   | 5.37        |
| Costanzo (2019)                                  | 3.54 (2.04, 6.13)   | 4.72        |
| Hudert (2019)                                    | 3.60 (1.99, 6.49)   | 4.50        |
| Lisboa (2020)                                    | 2.37 (1.47, 3.82)   | 5.13        |
| Subgroup, DL (l <sup>2</sup> = 0.0%, p = 0.617)  | > 2.45 (2.04, 2.94) | 34.89       |
| Heterogeneity between groups: p = 0.654          |                     |             |
| Overall, DL (l <sup>2</sup> = 74.5%, p = 0.000)  | > 2.33 (1.89, 2.88) | 100.00      |
| .0625 1  | 16                  |             |

| count and study (year)                           | Odds Ratio<br>(95% CI) | %<br>Weight |
|--|------------------------|-------------|
| >300   |                        |             |
| Hotta (2010)                                     | 2.02 (1.40, 2.92)      | 5.73        |
| Wang (2011)                                      | 1.72 (1.16, 2.54)      | 5.61        |
| Valenti (2012)                                   | 2.13 (1.40, 3.23)      | 5.46        |
| Bhatt (2013)                                     | 2.77 (1.61, 4.78)      | 4.74        |
| Rametta (2014)                                   | 2.30 (1.50, 3.52)      | 5.41        |
| Lee (2014)                                       | 1.71 (1.03, 2.82)      | 4.97        |
| Niu (2014)                                       | 5.77 (4.03, 8.27)      | 5.77        |
| Shang (2015)                                     | 1.09 (0.77, 1.54)      | 5.84        |
| Mazo (2018)                                      | 2.16 (1.40, 3.32)      | 5.37        |
| Yang (2018)                                      | 1.13 (0.69, 1.86)      | 5.01        |
| Wu (2020)  | 1.53 (1.06, 2.22)      | 5.73        |
| Delik (2020)                                     | 2.44 (1.46, 4.07)      | 4.92        |
| Subgroup, DL (I <sup>2</sup> = 80.2%, p = 0.000) | 2.02 (1.54, 2.65)      | 64.57       |
| <300   |                        |             |
| Vespasiani-Gentilucci (2016)                     | 2.11 (1.13, 3.96)      | 4.30        |
| Alam (2017)                                      | 2.51 (1.36, 4.65)      | 4.36        |
| Costanzo (2019)                                  | 3.54 (2.04, 6.13)      | 4.72        |
| Hudert (2019)                                    | 3.60 (1.99, 6.49)      | 4.50        |
| Narayanasamy (2020)                              | 6.21 (3.30, 11.70)     | 4.27        |
| Lisboa (2020)                                    | 2.37 (1.47, 3.82)      | 5.13        |
| Akkiz (2021)                                     | 2.96 (1.64, 5.33)      | 4.50        |
| Zhang (2021)                                     | 2.36 (1.11, 5.05)      | 3.65        |
| Subgroup, DL (I <sup>2</sup> = 18.7%, p = 0.282) | 3.01 (2.39, 3.80)      | 35.43       |
| Heterogeneity between groups; p = 0.029          |                        |             |
| Overall, DL (l <sup>2</sup> = 74.5%, p = 0.000)  | 2.33 (1.89, 2.88)      | 100.00      |
| .0625  | I I<br>1 16            |             |

# dominant allele model (CG+GG VS CC)

Figure 3. Continued

Results show that it can impact on the genetic susceptibility to NAFLD. It may help to understand the mechanism of transmission of NAFLD and to identify some risk groups. It shows that the rs738,409 polymorphism is significantly associated with NAFLD risk under homozygous, dominant, heterozygous, and recessive and allelic models. Subgroup analysis shows that the significant association between rs738,409 and NAFLD is not affected by sample size and race. The heterogeneity is still large. Meanwhile, the funnel plots demonstrated less publication bias. And the sensitivity analysis suggests that none of the individual studies affected the pooled OR of all the included studies. These data further enhanced the reliability and stability of the meta-analysis results.

The results showed that PNPLA3 gene is closely related to NAFLD. The reason may be that the increase of triglyceride content in liver is closely related to PNPLA3 gene. We speculate that the mechanism of PNPLA3 I148M mutation leading to increased liver triglyceride content may include the following aspects: PNPLA3 encodes adiponutrients located on the endoplasmic reticulum and lipid droplets in liver cells. PNPLA3 has a Pattin like domain at the N-terminal, showing hydrolase activity for glycerides (triacylglycerol, diacylglycerol D

| country and study (year)                         | Odds Ratio<br>(95% CI)   | %<br>Weigh |
|--|--------------------------|------------|
| Asian  |                          |            |
| Hotta (2010)                                     | 2.82 (2.02, 3.92)        | 6.49       |
| Wang (2011)                                      | 1.52 (1.00, 2.32)        | 6.1        |
| Bhatt (2013)                                     | 2.70 (1.12, 6.54)        | 4.0        |
| Lee (2014)                                       | 1.84 (1.12, 3.01)        | 5.79       |
| Niu (2014)                                       | <b>6.75 (4.73, 9.64)</b> | 6.3        |
| Shang (2015)                                     | 1.45 (0.92, 2.28)        | 5.9        |
| Alam (2017)                                      | 5.70 (1.62, 20.07)       | 2.7        |
| Yang (2018)                                      | 0.81 (0.50, 1.31)        | 5.8        |
| Wu (2020)  | 1.81 (1.11, 2.94)        | 5.8        |
| Narayanasamy (2020)                              | 3.28 (1.64, 6.56)        | 4.8        |
| Delik (2020)                                     | 2.11 (1.16, 3.82)        | 5.3        |
| Akkiz (2021)                                     | 2.46 (1.25, 4.83)        | 4.9        |
| Zhang (2021)                                     | 2.67 (1.23, 5.77)        | 4.5        |
| Subgroup, DL (I <sup>2</sup> = 82.2%, p = 0.000) | 2.29 (1.62, 3.25)        | 68.9       |
| Caucasian  |                          |            |
| Valenti (2012)                                   | 2.57 (1.30, 5.11)        | 4.9        |
| Rametta (2014)                                   | 2.63 (1.29, 5.36)        | 4.7        |
| Vespasiani-Gentilucci (2016)                     | 4.05 (1.57, 10.39)       | 3.8        |
| Mazo (2018)                                      | 2.59 (1.36, 4.95)        | 5.0        |
| Costanzo (2019)                                  | 6.26 (2.05, 19.14)       | 3.2        |
| Hudert (2019)                                    | 6.40 (2.86, 14.31)       | 4.3        |
| Lisboa (2020)                                    | 2.05 (1.03, 4.08)        | 4.8        |
| Subgroup, DL (I <sup>2</sup> = 15.7%, p = 0.311) | 3.13 (2.28, 4.30)        | 31.0       |
| Heterogeneity between groups: p = 0.194          |                          |            |
| Overall, DL (l <sup>2</sup> = 75.4%, p = 0.000)  | 2.56 (1.96, 3.35)        | 100.0      |
|  |                          |            |

NOTE: Weights and between-subgroup heterogeneity test are from random-effects model

recessive allele model (GG vs CC+CG)

| count and study (year)   | Odds Ratio<br>(95% CI)   | %<br>Weight |
|--|--------------------------|-------------|
| >300   |                          |             |
| Hotta (2010) -   | 2.82 (2.02, 3.92)        | 6.49        |
| Wang (2011)  | 1.52 (1.00, 2.32)        | 6.12        |
| Valenti (2012)   | 2.57 (1.30, 5.11)        | 4.90        |
| Bhatt (2013)   | 2.70 (1.12, 6.54)        | 4.05        |
| Rametta (2014)   | 2.63 (1.29, 5.36)        | 4.78        |
| Lee (2014)   | 1.84 (1.12, 3.01)        | 5.79        |
| Niu (2014)   | <b>6.75 (4.73, 9.64)</b> | 6.39        |
| Shang (2015)   | 1.45 (0.92, 2.28)        | 5.97        |
| Mazo (2018)  | 2.59 (1.36, 4.95)        | 5.08        |
| Yang (2018)  | 0.81 (0.50, 1.31)        | 5.85        |
| Wu (2020)  | 1.81 (1.11, 2.94)        | 5.83        |
| Delik (2020)   | 2.11 (1.16, 3.82)        | 5.32        |
| Subgroup, DL (I <sup>2</sup> = 83.0%, p = 0.000)                                   | 2.17 (1.53, 3.08)        | 66.59       |
| <300   |                          |             |
| Vespasiani-Gentilucci (2016)   | 4.05 (1.57, 10.39)       | 3.81        |
| Alam (2017)  | 5.70 (1.62, 20.07)       | 2.79        |
| Costanzo (2019) -  | 6.26 (2.05, 19.14)       | 3.21        |
| Hudert (2019)  | 6.40 (2.86, 14.31)       | 4.38        |
| Narayanasamy (2020)  | 3.28 (1.64, 6.56)        | 4.87        |
| Lisboa (2020)  | 2.05 (1.03, 4.08)        | 4.89        |
| Akkiz (2021)   | 2.46 (1.25, 4.83)        | 4.95        |
| Zhang (2021)   | 2.67 (1.23, 5.77)        | 4.52        |
| Subgroup, DL (l <sup>2</sup> = 8.1%, p = 0.368)                                    | 3.35 (2.48, 4.53)        | 33.41       |
| Heterogeneity between groups: p = 0.065  |                          |             |
| Overall, DL (l <sup>2</sup> = 75.4%, p = 0.000)                                    | 2.56 (1.96, 3.35)        | 100.00      |
| .0625 1  | 16                       |             |
| NOTE: Weights and between-subgroup heterogeneity test are from random-effects mode | 1                        |             |

Figure 3. Continued

and monoacylglycerol).<sup>[47]</sup> It plays a key role in the steady state of lipid metabolism. However, PNPLA3 148M acts in a "loss of function" way, resulting in low levels of glycerol phospholipid hydrolysis in the liver. And it inhibits lipid outflow to peripheral adipose tissue.<sup>[48]</sup> Therefore, the PNPLA3 148M variant depends on its interference with the balance of fat metabolism, leading to liver steatosis and related diseases; The content of liver fat in human body is positively correlated with the secretion of very low-density lipoprotein (VLDL) and apoB100.<sup>[49]</sup> When human liver fat content is the same, VLDL secretion speed of I148M allele carriers is slower. Moreover, McA-RH7777 cells overexpressing the I148M mutant protein showed an increase in intracellular triglyceride content. And apoB secretion is slow. There is no significant difference in the synthesis and stability of apoB mRNA. This suggests that the variation of PNPLA3 I148M can affect the secretion of apoB containing lipoproteins and the esterification of VLDL.<sup>[50]</sup> Therefore, it can promote the increase of triglyceride content in the liver; PNPLA3 I148M promotes the synthesis of liver triglycerides by enhancing the role of lysophosphatidic acid acyltransferase;<sup>[51]</sup> and The protein inactivated on the lipid droplet surface may prevent the hydrolysis of triglycerides by restricting the lipid droplet pathway. It causes the accumulation of triglyceride levels in the liver.<sup>[52]</sup> The specific function

| - |
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| - |
| _ |

| country and study (year)                         | (95% CI)                              | %<br>Weight |
|--|---------------------------------------|-------------|
| Asian  |                                       |             |
| Hotta (2010)                                     | - 1.46 (0.98, 2.16)                   | 6.38        |
| Wang (2011)                                      | — 1.61 (1.06, 2.42)                   | 6.18        |
| Bhatt (2013)                                     | 2.91 (1.53, 5.52)                     | 4.12        |
| _ee (2014)                                       | 1.45 (0.85, 2.47)                     | 4.97        |
| Niu (2014)                                       | 3.31 (2.26, 4.87)                     | 6.47        |
| Shang (2015)                                     | 1.00 (0.69, 1.44)                     | 6.64        |
| Alam (2017)                                      | 1.94 (1.01, 3.71)                     | 4.06        |
| Yang (2018)                                      | - 1.32 (0.76, 2.30)                   | 4.83        |
| Wu (2020)  | 1.37 (0.93, 2.02)                     | 6.44        |
| Narayanasamy (2020)                              | 5.35 (2.68, 10.68)                    | 3.77        |
| Delik (2020)                                     | 2.08 (1.14, 3.81)                     | 4.41        |
| Akkiz (2021)                                     | 2.43 (1.20, 4.93)                     | 3.66        |
| Zhang (2021)                                     | 1.83 (0.81, 4.13)                     | 3.05        |
| Subgroup, DL (I <sup>2</sup> = 67.7%, p = 0.000) | > 1.88 (1.46, 2.41)                   | 64.97       |
| Caucasian  |                                       |             |
| /alenti (2012)                                   | 1.90 (1.22, 2.95)                     | 5.89        |
| Rametta (2014)                                   | <ul> <li>2.07 (1.33, 3.24)</li> </ul> | 5.83        |
| /espasiani-Gentilucci (2016)                     | 1.52 (0.74, 3.08)                     | 3.64        |
| Mazo (2018)                                      | 1.82 (1.15, 2.89)                     | 5.69        |
| Costanzo (2019) -                                | 2.90 (1.64, 5.14)                     | 4.66        |
| Hudert (2019)                                    | 2.58 (1.37, 4.86)                     | 4.16        |
| Lisboa (2020)                                    | <ul> <li>2.20 (1.31, 3.68)</li> </ul> | 5.15        |
| Subgroup, DL (I <sup>2</sup> = 0.0%, p = 0.805)  | > 2.08 (1.71, 2.53)                   | 35.03       |
| Heterogeneity between groups: p = 0.520          |                                       |             |
| Overall, DL (I <sup>2</sup> = 54.9%, p = 0.002)  | > 1.93 (1.63, 2.30)                   | 100.00      |
| 125 1  | 1                                     |             |

| count and study (year)                           |            | (95% CI)           | Weight   |
|--|------------|--------------------|----------|
| >300   |            |                    |          |
| Hotta (2010)                                     |            | 1.46 (0.98, 2.16   | ) 6.38   |
| Wang (2011)                                      |            | 1.61 (1.06, 2.42   | ) 6.18   |
| Valenti (2012)                                   |            | 1.90 (1.22, 2.95   | ) 5.89   |
| Bhatt (2013)                                     |            | 2.91 (1.53, 5.52   | ) 4.12   |
| Rametta (2014)                                   |            | 2.07 (1.33, 3.24   | 5.83     |
| Lee (2014)                                       | -          | 1.45 (0.85, 2.47   | ) 4.97   |
| Niu (2014)                                       |            | 3.31 (2.26, 4.87   | 6.47     |
| Shang (2015)                                     | -          | 1.00 (0.69, 1.44   | 6.64     |
| Mazo (2018)                                      |            | 1.82 (1.15, 2.89   | ) 5.69   |
| Yang (2018)                                      |            | 1.32 (0.76, 2.30   | 4.83     |
| Wu (2020)  |            | 1.37 (0.93, 2.02   | ) 6.44   |
| Delik (2020)                                     |            | 2.08 (1.14, 3.81   | ) 4.41   |
| Subgroup, DL (I <sup>2</sup> = 58.9%, p = 0.005) | $\diamond$ | 1.73 (1.41, 2.13   | 67.85    |
| <300   |            |                    |          |
| Vespasiani-Gentilucci (2016)                     | -          | 1.52 (0.74, 3.08   | ) 3.64   |
| Alam (2017)                                      | *          | 1.94 (1.01, 3.71   | ) 4.06   |
| Costanzo (2019)                                  |            | 2.90 (1.64, 5.14   | ) 4.66   |
| Hudert (2019)                                    |            | 2.58 (1.37, 4.86   | ) 4.16   |
| Narayanasamy (2020)                              |            | 5.35 (2.68, 10.6   | B) 3.77  |
| Lisboa (2020)                                    |            | 2.20 (1.31, 3.68   | ) 5.15   |
| Akkiz (2021)                                     |            | 2.43 (1.20, 4.93   | ) 3.66   |
| Zhang (2021)                                     |            | - 1.83 (0.81, 4.13 | ) 3.05   |
| Subgroup, DL (I <sup>2</sup> = 14.7%, p = 0.315) | $\diamond$ | 2.44 (1.90, 3.12   | ) 32.15  |
| Heterogeneity between groups: p = 0.038          |            |                    |          |
| Overall, DL (I <sup>2</sup> = 54.9%, p = 0.002)  | $\diamond$ | 1.93 (1.63, 2.30   | ) 100.00 |
|  |            |                    |          |

# heterozygote model (CG vs CC)

Figure 3. Continued

of PNPLA3 may vary from target organ to target organ, and it is still controversial. Whether the main role of triglycerides in the liver is reflected in the hydrolysis process or the synthesis process is still uncertain.

This article also has some limitations. First, the literature we included may not be comprehensive, only those published case-control studies were included. Secondly, most of the research objects are Asians and Caucasians, and there are few articles by Spaniards. Third, NAFLD is a multifactorial disease, so the possibility of the relationship between gene and gene or between gene and environment needs to be further considered. The sample size is relatively small. We need larger, multi-ethnic, high-quality articles to explore the relationship between rs738,409 and NAFLD.

At present, there is no clinical application of SNP sites as indicators for disease judgment and prognosis prediction. The main reason is that the odds ratio of SNP sites is low and the ability to judge diseases is weak.<sup>[53]</sup> Therefore, in order to improve the predictive ability of the incidence risk of liver cancer, it is recommended to include PNPLA3 rs738,409 and other В

| country and study (year)   | Odds Ratio<br>(95% Cl) Weig   |
|--|---|
| Asian  |   |
| Hotta (2010)   | 3.63 (2.36, 5.57) 5.9   |
| Wang (2011)  | 2 03 (1 23 3 35) 57   |
| Wally (2011)   |   |
| Bhatt (2013)   | 2.49 (1.02, 6.09) 4.5   |
| Lee (2014)   | 2.35 (1.27, 4.34) 5.4   |
| Niu (2014)   | 14 41 (9 23 22 49) 5 9  |
| Change (2015)  |   |
| Shang (2015)   | 1.45 (0.88, 2.38) 5.7   |
| Alam (2017)  | 7.70 (2.11, 28.07) 3.3  |
| Yang (2018)  | 0.95 (0.53, 1.69) 5.5   |
| W(((2020))   | 2 18 (1 27 3 73) 5 6  |
| Wd (2020)  | 2.10 (1.27, 3.73) 3.0   |
| Narayanasamy (2020)  | 7.98 (3.57, 17.86) 4.7  |
| Delik (2020)   | 2.89 (1.52, 5.49) 5.3   |
| Akkiz (2021)   | 3.56 (1.72, 7.36) 5.0   |
| Zhang (2021)   |   |
| znang (2021)   | 3.89 (1.52, 9.93) 4.3   |
| Subgroup, DL (I <sup>e</sup> = 85.8%, p = 0.000)   | 3.16 (2.01, 4.98) 67.2  |
| Caucasian  |   |
| Valenti (2012)   | 3.48 (1.69, 7.16) 5.0   |
| Rametta (2014)   | 3 73 (1 76 7 87) 4 0  |
| Vennenieni Cantilueni (2016)   |   |
| vespasiani-Gentilucci (2016)   | 4.65 (1.75, 12.35) 4.2  |
| Mazo (2018)  | 3.56 (1.79, 7.09) 5.1   |
| Costanzo (2019)  | 10.69 (3.35. 34.07) 3.7   |
| Hudert (2019)  |   |
| 10001 (2018)   | 10.19 (4.22, 24.59) 4.5   |
| Lisboa (2020)  | 2.91 (1.41, 6.02) 5.0   |
| Subgroup, DL (1 <sup>2</sup> = 24.5%, p = 0.242)   | 4.44 (3.11, 6.32) 32.7  |
| Heterogeneity between groups: p = 0.250  |   |
| Overall, DL (I <sup>2</sup> = 80.1%, p = 0.000)  | 3.59 (2.56, 5.04) 100.0   |
| .03125   | i 32<br>fects model   |
| .03125 · ·   | t 32<br>tects model<br>Odds Ratio   |
| .03125 *   | i 32<br>fects model<br>Odds Ratio<br>(95% CI) Weig  |
| .03125 · ·<br>NOTE: Weights and between-subgroup heterogeneity test are from random-eff<br>count and study (year)<br>>300  | Odds Ratio<br>(95% CI) Weig   |
| .03125 · · · · · · · · · · · · · · · · · · ·   | 1 32<br>tects model<br>Odds Ratio (95% Ci) Weig<br>→ 3.63 (2.36, 5.57) 5.5  |
| .03125 * NOTE: Weights and between-subgroup heterogeneity test are from random-eff count and study (year) >300 Hotta (2010) Wang (2011)  | Odds Ratio<br>(95% Cl) Weig   |
| .03125 * NOTE: Weights and between-subgroup heterogeneity test are from random-eff count and study (year) >300 Hotta (2010) Wang (2011) Valenti (2012)   | Odds Ratio<br>(95% Cl) Weig   |
| .03125 * NOTE: Weights and between-subgroup heterogeneity test are from random-eff count and study (year) >300 Hotta (2010) Wang (2011) Valenti (2012) Detat (201 | Odds Ratio<br>(95% Cl) Weig   |
| .03125 ** NOTE: Weights and between-subgroup heterogeneity test are from random-eff count and study (year) >300 Hotta (2010) Wang (2011) Valenti (2012) Bhatt (2013)   | Odds Ratio<br>(95% Cl) Weig   |
| .03125 ** NOTE: Weights and between-subgroup heterogeneity test are from random-eff count and study (year) >300 Hotta (2010) Wang (2011) Valenti (2012) Bhatt (2013) Rametta (2014)  | Odds Ratio<br>(95% Cl) Weig   |
| .03125 * NOTE: Weights and between-subgroup heterogeneity test are from random-eff count and study (year) >300 Hotta (2010) Wang (2011) Valenti (2012) Bhatt (2013) Rametta (2014) Lee (2014) Lee (2014)   | Odds Ratio<br>(95% Cl) Weig   |
| .03125 · · · · · · · · · · · · · · · · · · ·   | Odds Ratio<br>(95% Cl) Weig   |
| .03125 *** NOTE: Weights and between-subgroup heterogeneity test are from random-eff count and study (year) >300 Hotta (2010) Wang (2010) Wang (2011) Valenti (2012) Bhatt (2013) Rametta (2014) Lee (2014) Lee (2014) Niu (2014)  | Odds Ratio<br>(95% Cl) Weig   |
| .03125 *** NOTE: Weights and between-subgroup heterogeneity test are from random-eff count and study (year) >300 Hotta (2010) Wang (2011) Valenti (2012) Bhatt (2013) Rametta (2014) Lee (2014) Niu (2014) Shang (2015)  | Odds Ratio<br>(95% Cl)         0           •         3.63 (2.36, 5.57)         5.6           •         3.63 (2.36, 5.57)         5.7           •         3.63 (2.36, 5.57)         5.6           •         3.48 (1.69, 7.16)         5.7           •         3.48 (1.99, 7.16)         5.7           •         3.73 (1.76, 7.87)         4.5           •         •         2.35 (1.27, 4.34)           •         •         1.44 (0.28, 2.249)         5.7           •         •         1.44 (0.28, 2.249)         5.7  |
| .03125<br>NOTE: Weights and between-subgroup heterogeneity test are from random-eff<br>count and study (year)<br>>300<br>Hotta (2010)<br>Wang (2010)<br>Wang (2011)<br>Valenti (2012)<br>Bhatt (2014)<br>Lee (2014)<br>Lee (2014)<br>Niu (2014)<br>Shang (2015)<br>  | Odds Ratio<br>(95% Cl)         9           ••••         3.63 (2.36, 5.57)         5.6           ••••         2.03 (1.23, 3.35)         5.7           ••••         2.03 (1.23, 3.35)         5.7           ••••         2.03 (1.23, 3.35)         5.7           ••••         2.49 (1.02, 6.09)         4.5           ••••         2.35 (1.27, 4.34)         5.6           ••••         1.44 (0.88, 2.38)         5.7           ••••         3.56 (1.27, 4.34)         5.5           ••••         3.56 (1.27, 1.34)         5.5           ••••         3.56 (1.27, 1.34)         5.5           ••••         3.56 (1.27, 1.34)         5.5           ••••         3.56 (1.27, 1.34)         5.5  |
| .03125 · · · · · · · · · · · · · · · · · · ·   | Odds Ratio<br>(95% Cl)         0           ••••••••••••••••••••••••••••••••••••   |
| .03125  NOTE: Weights and between-subgroup heterogeneity test are from random-eff count and study (year) >300 Hotta (2010) Wang (2010) Valenti (2012) Bhatt (2013) Rametta (2014) Lee (2014) Lee (2014) Niu (2014) Shang (2015) Mazo (2018) Yang (2018)  | Odds Ratio<br>(95% Cl)         Weig   |
| .03125 *** NOTE: Weights and between-subgroup heterogeneity test are from random-eff count and study (year) >300 Hotta (2010) Wang (2011) Valenti (2012) Bhatt (2013) Rametta (2014) Lee (2014) Niu (2014) Shang (2015) Mazo (2018) Yang (2018) Yang (2018) Yang (2018)  | Odds Ratio<br>(95% Cl)         0           ••••••••••••••••••••••••••••••••••••   |
| .03125  NOTE: Weights and between-subgroup heterogeneity test are from random-eff count and study (year) >300 Hotta (2010) Wang (2011) Valenti (2012) Bhatt (2013) Rametta (2014) Lee (2014) Lee (2014) Shang (2015) Mazo (2018) Yang (2018) Yang (2020) Delik (2020)  | Odds Ratio<br>(95% Cl)         Weig   |
| .03125 • • • • • • • • • • • • • • • • • • •   | Odds Ratio<br>(95% Cl)         9           3.63 (2.36, 5.57)         5.6           2.03 (1.23, 3.36)         5.7           3.63 (2.36, 5.57)         5.6           2.03 (1.23, 3.36)         5.7           3.48 (1.69, 7.16)         5.6           2.49 (1.02, 6.09)         4.5           3.73 (1.76, 7.87)         4.5           2.35 (1.27, 4.34)         5.4           1.45 (0.88, 2.38)         5.7           3.56 (1.79, 7.09)         5.1           2.18 (1.27, 3.73)         5.6           2.89 (1.52, 5.49)         5.3           2.89 (1.52, 5.49)         5.3           2.89 (1.52, 5.49)         5.3  |
| .03125 • • • • • • • • • • • • • • • • • • •   | Odds Ratio<br>(95% Cl)         0           363 (2.36, 5.57)         5.6           2.03 (1.23, 3.35)         5.7           3.68 (2.36, 5.57)         5.6           2.03 (1.23, 3.35)         5.7           3.48 (1.69, 7.16)         5.6           2.35 (1.27, 4.34)         5.4           3.56 (1.79, 7.09)         5.1           3.56 (1.79, 7.09)         5.1           0.95 (0.53, 1.69)         5.7           2.89 (1.52, 5.49)         5.3           2.83 (1.82, 4.40)         64.5  |
| .03125   | Odds Ratio<br>(95% Cl)         9           •         3.63 (2.36, 5.57)         5.6           2.03 (1.23, 3.35)         5.7           •         3.68 (2.36, 5.57)         5.6           •         2.03 (1.23, 3.35)         5.7           •         3.48 (1.69, 7.16)         5.0           •         2.35 (1.27, 4.34)         5.4           •         1.45 (0.48, 2.38)         5.7           •         1.45 (0.48, 2.38)         5.7           •         1.45 (0.48, 2.38)         5.7           •         1.45 (0.48, 2.38)         5.7           •         1.45 (0.48, 2.38)         5.7           •         1.45 (0.48, 2.38)         5.7           •         1.45 (0.48, 2.38)         5.7           •         1.45 (0.48, 2.38)         5.7           •         1.45 (0.48, 2.38)         5.7           •         2.18 (1.27, 3.73)         5.6           •         2.89 (1.52, 5.49)         5.3           •         2.83 (1.82, 4.40)         64.5   |
| .03125 • • • • • • • • • • • • • • • • • • •   | Odds Ratio<br>(95% Cl)         Weig           3.63 (2.36, 5.57)         5.6           2.03 (1.23, 3.35)         5.7           3.48 (1.69, 7.16)         5.6           2.37 (1.76, 7.87)         4.5           3.58 (1.27, 4.34)         5.6           3.56 (1.27, 4.34)         5.5           3.56 (1.77, 7.97)         4.5           2.38 (1.27, 4.34)         5.5           4.65 (1.27, 5.9)         5.2           4.65 (1.52, 5.49)         5.5           2.89 (1.52, 5.49)         5.5           2.83 (1.82, 4.40)         64.5           4.65 (1.75, 12.35)         4.2  |
| .03125 · · · · · · · · · · · · · · · · · · ·   | Odds Ratio<br>(95% Cl)         Weig   |
| .03125 • • • • • • • • • • • • • • • • • • •   | Odds Ratio<br>(95% Cl)         Weig           3.63 (2.36, 5.57)         5.6           2.03 (1.23, 3.35)         5.7           3.48 (1.69, 7.16)         5.6           2.37 (1.76, 7.87)         4.5           3.58 (1.27, 4.34)         5.6           3.56 (1.27, 4.34)         5.6           1.45 (0.88, 2.38)         5.7           3.56 (1.77, 7.97)         4.5           2.38 (1.82, 5.49)         5.5           2.83 (1.82, 5.49)         5.5           2.83 (1.82, 5.49)         5.5           2.83 (1.82, 4.40)         64.5           4.65 (1.75, 12.35)         4.2           4.65 (1.75, 12.35)         4.2           1.68 (3.63 (2.36, 2.407)         3.3           1.68 (3.63 (2.36, 2.407)         3.3           1.68 (3.63 (2.36, 2.407)         3.3           1.68 (3.63 (2.36, 2.407)         3.3           1.68 (3.63 (2.36, 2.407)         3.3           1.68 (3.63 (2.36, 2.407)         3.3           1.68 (3.63 (2.36, 2.407)         3.3           1.68 (3.63 (2.36, 2.407)         3.3           1.68 (3.63 (2.36, 2.407)         3.3           1.68 (3.63 (2.36, 2.407)         3.3           1.68 (3.63 (2.36, 2.407)         3.3 |
| .03125 NOTE: Weights and between-subgroup heterogeneity test are from random-eff count and study (year) >300 Hotta (2010) Wang (2011) Valenti (2012) Bhatt (2013) Rametta (2014) Lee (2014) Niu (2014) Shang (2015) Mazo (2018) Yang (2018) Yang (2018) Yang (2018) Valenti (2020) Delik (2020) Delik (2020) Subgroup, DL (I <sup>2</sup> = 85.7%, p = 0.000) <300 Vespasiani-Gentilucci (2016) Alam (2017) Costanzo (2019)  | Odds Ratio<br>(95% CI)         Weig   |
| .03125 • • • • • • • • • • • • • • • • • • •   | Odds Ratio<br>(95% Cl)         Weig           3.63 (2.36, 5.57)         5.6           2.03 (1.23, 3.35)         5.7           3.48 (1.69, 7.16)         5.6           2.03 (1.23, 3.35)         5.7           3.48 (1.69, 7.16)         5.6           2.37 (1.76, 7.87)         4.5           3.73 (1.76, 7.87)         4.5           2.35 (1.27, 4.34)         5.6           2.35 (1.27, 4.34)         5.6           2.35 (1.27, 7.33)         5.6           2.35 (1.27, 4.34)         5.4           1.45 (0.88, 2.38)         5.7           3.56 (1.27, 3.73)         5.6           2.83 (1.82, 5.49)         5.5           2.83 (1.82, 5.49)         5.5           2.83 (1.82, 5.40)         64.5           4.65 (1.75, 12.35)         4.2           4.65 (1.75, 12.35)         4.2           4.65 (1.75, 12.35)         4.2           4.65 (1.75, 12.35)         4.2           4.65 (1.75, 12.35)         4.2           4.65 (1.75, 12.35)         4.2           4.65 (1.75, 12.25, 5.49)         5.3           4.65 (1.75, 12.35)         4.2           4.65 (1.75, 12.35)         4.2           4.65 (1.75, 12.25, 9)         4.5 </td                |
| .03125 • • • • • • • • • • • • • • • • • • •   | Odds Ratio<br>(95% Cl)         Weig   |
| .03125 • • NOTE: Weights and between-subgroup heterogeneity test are from random-eff count and study (year) >300 Hotta (2010) Wang (2011) Valenti (2012) Bhatt (2013) Rametta (2014) Lee (2014) Niu (2014) Shang (2015) Mazo (2018) Wu (2020) Delik (2020) Delik (2020) Delik (2020) Subgroup, DL (I <sup>2</sup> = 85.7%, p = 0.000) <300 Vespasiani-Gentilucci (2016) Alam (2017) Costanzo (2019) Hudert (2019) Narayanasamy (2020) Lichera (2020)   | Odds Ratio<br>(95% Cl)         Weig           3.63 (2.36, 5.57)         5.6           2.03 (1.23, 3.35)         5.7           3.48 (1.69, 7.16)         5.6           2.03 (1.23, 3.35)         5.7           3.48 (1.69, 7.16)         5.6           2.37 (1.76, 7.87)         4.5           3.73 (1.76, 7.87)         4.5           2.38 (1.27, 4.34)         5.6           2.35 (1.27, 4.34)         5.6           3.56 (1.27, 7.33)         5.6           2.83 (1.82, 2.49)         5.5           2.83 (1.82, 5.49)         5.5           2.83 (1.82, 5.40)         64.5           4.65 (1.75, 12.35)         4.2           7.70 (2.11, 28.07)         3.3           10.19 (4.22, 24.59)         4.5           7.70 (2.11, 28.07)         3.7           10.19 (4.22, 24.59)         4.5           7.70 (2.11, 28.07)         3.7           10.19 (4.22, 24.59)         4.5           7.70 (2.11, 28.07)         3.7           7.70 (2.11, 28.07)         3.7           7.70 (2.11, 28.07)         3.7           7.70 (2.11, 28.07)         3.7           7.70 (2.11, 28.07)         3.7           7.70 (2.11, 28.07)         3.7                        |
| .03125 • • • • • • • • • • • • • • • • • • •   | Odds Ratio<br>(95% Cl)         Weig           0         3.63 (2.36, 5.57)         5.6           2.03 (1.23, 3.35)         5.7           3.68 (2.36, 5.57)         5.6           2.03 (1.23, 3.35)         5.7           3.48 (1.69, 7.16)         5.6           2.35 (1.27, 4.34)         5.4           1.45 (0.48, 2.38)         5.7           3.56 (1.79, 7.09)         5.1           1.45 (0.48, 2.38)         5.7           2.35 (1.27, 4.34)         5.4           1.45 (0.48, 2.38)         5.7           3.56 (1.79, 7.09)         5.1           0.95 (0.53, 1.69)         5.5           2.83 (1.82, 4.40)         64.5           4.65 (1.75, 12.35)         4.2           7.70 (2.11, 28.07)         3.3           10.69 (3.35, 34.07)         3.7           10.19 (4.22, 24.59)         4.5           7.98 (3.57, 17.86)         4.5           7.91 (1.41, 6.02)         5.0   |
| .03125 ************************************  | Odds Ratio<br>(95% Cl)         Weig           3.63 (2.36, 5.57)         5.6           2.03 (1.23, 3.35)         5.7           3.48 (1.69, 7.16)         5.6           2.37 (1.76, 7.87)         4.5           3.73 (1.76, 7.87)         4.5           2.35 (1.27, 4.34)         5.6           1.45 (0.88, 2.38)         5.7           3.56 (1.79, 7.09)         5.5           2.38 (1.52, 5.49)         5.5           2.83 (1.52, 5.49)         5.5           2.83 (1.52, 5.49)         5.5           2.83 (1.52, 5.49)         5.5           2.83 (1.52, 5.49)         5.5           2.83 (1.52, 5.49)         5.5           2.83 (1.52, 5.49)         5.5           2.83 (1.52, 5.49)         5.5           2.83 (1.52, 5.49)         5.4           7.70 (2.11, 28.07)         3.3           10.69 (3.55, 34.07)         3.7           10.19 (4.22, 24.59)         4.2           7.98 (3.57, 17.86)         4.7           7.91 (1.41, 6.02)         5.0           3.56 (1.72, 7.36)         5.5   |
| .03125 • • • • • • • • • • • • • • • • • • •   | Odds Ratio<br>(95% CI)         Weig   |
| .03125 • • • • • • • • • • • • • • • • • • •   | Odds Ratio<br>(95% Cl)         Weig           0         3.63 (2.36, 5.57)         5.6<br>(95% Cl)           0         3.63 (2.36, 5.57)         5.6<br>(2.03 (1.23, 3.35)           3.48 (1.69, 7.16)         5.0<br>(2.49 (1.02, 6.09)         4.5<br>(2.35 (1.27, 4.34)           3.73 (1.76, 7.87)         4.4<br>(1.44, 11 (9.23, 22.49)         5.5<br>(2.35 (1.27, 4.34)           1.45 (0.48, 2.38)         5.7<br>(3.56 (1.79, 7.09)         5.1<br>(2.98 (1.52, 5.49)           2.89 (1.52, 5.49)         5.3<br>(2.83 (1.82, 4.40)         64.5<br>(1.72, 7.38)           1.09 (4.22, 24.59)         4.5<br>(1.41, 6.02)         5.4<br>(3.75, 17.36)           1.09 (3.35, 34.07)         3.7<br>(1.0.19 (4.22, 24.59)         4.5<br>(3.58 (1.72, 7.38)           1.05 (3.38 (1.52, 9.93)         4.3<br>(3.88 (1.52, 9.93)         4.3<br>(3.63 (3.72, 7.67)   |
| .03125 • • • • • • • • • • • • • • • • • • •   | Odds Ratio<br>(95% C1)         Weig           0         3.63 (2.36, 5.57)         5.6<br>(95% C1)           0         3.63 (2.36, 5.57)         5.6<br>(2.03 (1.23, 3.35)           3.48 (1.69, 7.16)         5.0<br>(2.49 (1.02, 6.09)         4.5<br>(2.35 (1.27, 4.34)           0         3.73 (1.76, 7.87)         4.8<br>(1.84, 10, 9.2, 22.49)         5.7<br>(1.76, 7.87)           1.45 (0.48, 2.38)         5.7<br>(3.56 (1.79, 7.09)         5.1<br>(2.98 (1.52, 5.49)         5.2<br>(2.83 (1.82, 4.40)           4.65 (1.75, 12.35)         4.2<br>(1.99 (3.35, 34.07)         3.7<br>(1.0.19 (4.22, 24.59)         4.2<br>(3.56 (1.72, 7.36)           10.59 (3.55, 34.07)         3.7<br>(1.0.19 (4.22, 24.59)         4.2<br>(3.56 (1.72, 7.36)         4.2<br>(3.56 (1.72, 7.36)           10.59 (3.55, 34.07)         3.2<br>(1.41, 6.02)         5.3<br>(3.89 (1.52, 9.93)         4.3<br>(3.5)           10.59 (3.55, 34.07)         3.5<br>(3.89 (1.52, 9.93)         4.3<br>(3.5)         5.34 (3.72, 7.67)   |
| .03125 NOTE: Weights and between-subgroup heterogeneity test are from random-eff count and study (year) >300 Hotta (2010) Wang (2011) Valenti (2012) Bhatt (2013) Rametta (2014) Lee (2014) Shang (2015) Mazo (2018) Wu (2020) Delik (2020) Subgroup, DL (I <sup>2</sup> = 85.7%, p = 0.000) <300 Vespasiani-Gentilucci (2016) Alam (2017) Costanzo (2019) Hudert (2019) Narayanasamy (2020) Lisboa (2020) Narayanasamy (2020) Lisboa (2020) Akkiz (2021) Zhang (2021) Subgroup, DL (I <sup>2</sup> = 22.9%, p = 0.247) Heterogeneity between groups: p = 0.029 Over M. BU (2020)  | Odds Ratio<br>(95% Cl)         Weig           3.63 (2.36, 5.57)         5.6           2.03 (1.23, 3.35)         5.7           3.48 (1.69, 7.16)         5.7           2.33 (1.23, 3.35)         5.7           3.48 (1.69, 7.16)         5.6           2.33 (1.27, 4.34)         5.4           1.45 (0.88, 2.38)         5.7           3.56 (1.79, 7.09)         5.5           2.35 (1.77, 4.34)         5.4           1.45 (0.88, 2.38)         5.7           3.55 (1.79, 7.09)         5.5           2.83 (1.82, 5.49)         5.5           2.83 (1.82, 5.49)         5.5           2.83 (1.82, 5.40)         64.5           7.70 (2.11, 28.07)         3.3           10.99 (3.5, 54.07)         3.7           10.99 (3.5, 54.07)         3.7           10.99 (3.5, 54.07)         3.7           10.99 (3.5, 54.07)         3.5           3.89 (1.52, 9.93)         4.3           3.53 (1.72, 7.36)         5.3           3.89 (1.52, 9.93)         4.3           3.53 (1.72, 7.36)         5.34 (3.77, 35.6)           3.89 (1.52, 9.93)         4.3           3.53 (1.72, 7.76)         3.50 (1.72, 7.76)           3.89 (1.52, 9.93)         4         |

homozygote model (GG vs CC)

NOTE: Weights and between-subgroup heterogeneity test are from random-effects model

Figure 3. Continued

related gene SNP analysis. Then, a more accurate prediction and evaluation model of NAFLD disease may be established.

#### 5. Conclusion

PNPLA3 rs738,409 may play a significant role in increasing risk of NAFLD.

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# **Author contributions**

Conceptualization: Ye Zhao. Data curation: Yan Zhao, Jingchao Ma. Formal analysis: Yan Zhao, Wenli Zhao, Maeda Toshiyoshi. Funding acquisition: Ye Zhao. Investigation: Jingchao Ma. Methodology: Wenli Zhao, Ye Zhao. Resources: Yan Zhao, Jingchao Ma, Maeda Toshiyoshi. Software: Yan Zhao. Supervision: Ye Zhao. Validation: Ye Zhao. Visualization: Ye Zhao.



ALT under homozygous model



## AST under homozygous model

Figure 4. ALT and AST under the homozygous model. ALT = alanine aminotransferase, AST = aspartate aminotransferase.

Writing – original draft: Yan Zhao, Ye Zhao. Writing – review & editing: Yan Zhao, Wenli Zhao, Ye Zhao.

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Figure 5. Sensitivity analysis of the dominant model.



Figure 6. Begg funnel plot of dominant model.

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