Systematic Review and Meta-Analysis

Medicine

OPEN

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[a], Wenli Zhao, MD, PhD[b,c], Jingchao Ma, MPH[d], Maeda Toshiyoshi, MM[e], Ye Zhao, MD, PhD, MBA[f,*]

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 $P < .10$, $I^2 > 50\%$. 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

This project was supported by the Krirk University Science Foundation for Young Scholars: 2022 (107).

The authors declare no conflict of interests regarding the publication of this paper.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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

How to cite this article: Zhao Y, Zhao W, Ma J, Toshiyoshi M, Zhao Y. 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. Medicine 2023;102:10(e33110).

Received: 3 January 2023 / Received in final form: 3 February 2023 / Accepted: 7 February 2023

http://dx.doi.org/10.1097/MD.0000000000033110

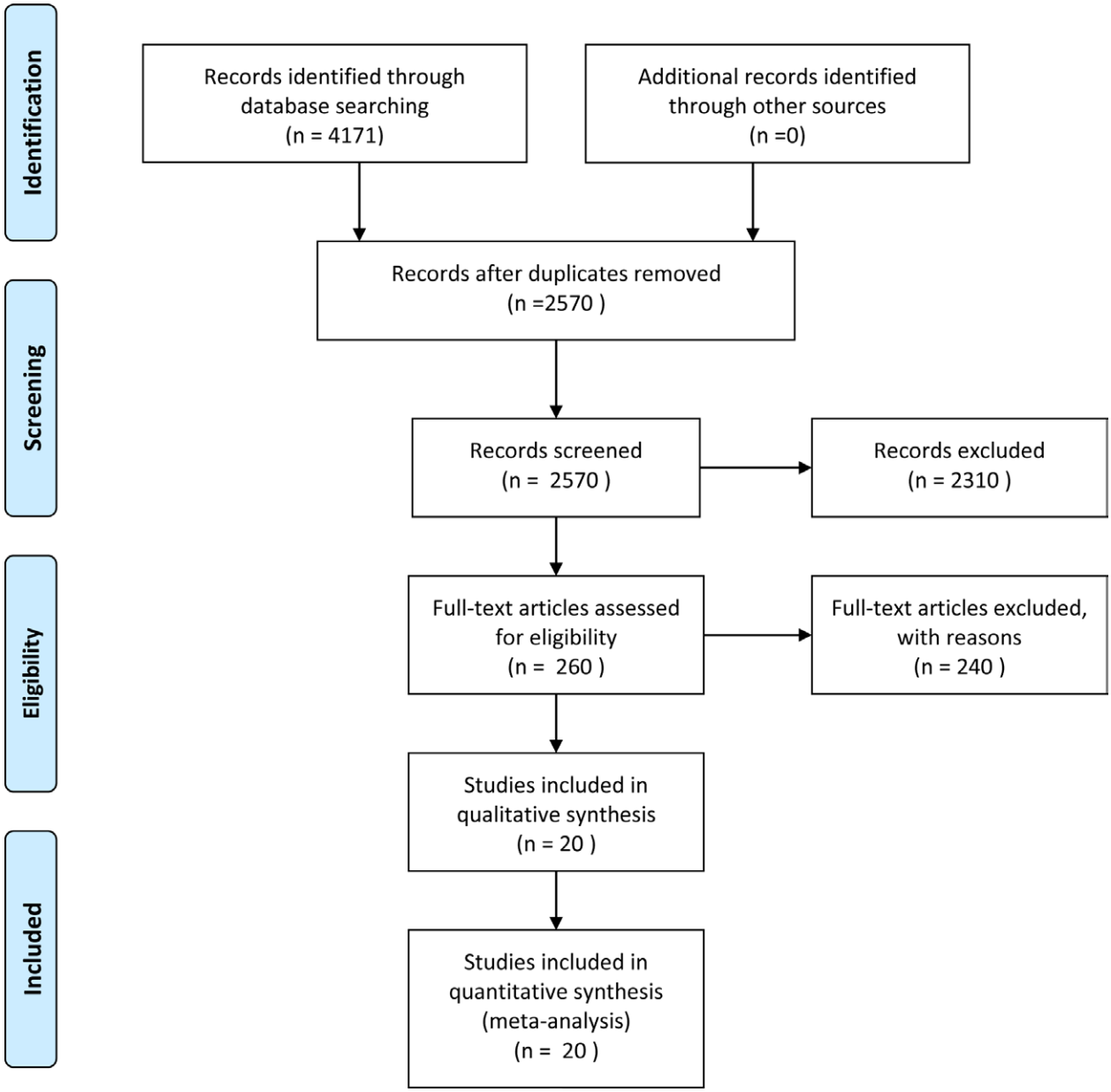


Figure 1. PRISMA 2009 flow diagram. PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of liver disease.[1] It is a clinical syndrome characterized by diffuse hepatocyte steatosis, excluding excessive drinking and other clear liver damaging factors.[2] Due to changes in lifestyle, the prevalence of NAFLD increases at an alarming rate.[3] By 2018, the prevalence of NAFLD worldwide was 24.4%, including 20% to 30% in western countries and 5% to 18% in Asian countries.[4] Even in Africa, where the lowest prevalence is, the incidence rate is 14%.[5] 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.[6] NASH can evolve into liver fibrosis, liver cirrhosis and finally into hepatocellular carcinoma. Hepatocellular carcinoma is the most serious consequence of NAFLD.[7] The pathogenesis of NAFLD is not clear.[8] Sensitivity of the liver to the damage increases insulin resistance.[9] 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.[10] 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.[11] Hepatocyte injury is caused by the secretion of inflammatory factors, oxidative stress, gut dysbiosis and mitochondrial dysfunction.[12] Furthermore, it deteriorates into NASH, liver fibrosis, cirrhosis.[13] No specific drug has been authorized for the treatment of NAFLD.[14] Health education and lifestyle interventions are therefore recommended as its first-line treatments.[15] 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 studies included in the meta-analysis.

First author	Yr	Country	Age	Gender (men/women)	NAFLD diagnosis	Source of control	Genotyping method
Hotta[25]	2010	Japan	N:51.7 ± 15.0 C:47.2 ± 14.8	304/527	LB	HB	TaqMan
Wang[26]	2011	China	N:48.1 ± 12.95 C:45.4 ± 15.93	407/472	US	HB	TaqMan
Valenti[27]	2012	Italy	N:49.5 ± 12 C:48 ± 12	314/87	LB	HB	Real-time PCR
Bhatt[28]	2013	India	N:38.2 ± 7.0 C:37.1 ± 6.9	NA	US	HB	Real-time PCR
Rametta[29]	2014	Italy	N:49.7 ± 12.1 C:47.7 ± 12.1	314/83	LB	HB	TaqMan
Lee[30]	2014	Korea	N:44.1 ± 15.5 C:45.3 ± 10.6	161/178	US	HB	TaqMan
Niu[31]	2014	China	N:49.76 ± 16.17 C:47.69 ± 15.86	373/426	US	HB	ABI sequencer
Shang[32]	2015	China	N:11.81 ± 2.20 C:11.44 ± 2.99	453/574	US	PB	Mass ARRAY
Vespasiani-Gentilucci[33]	2016	Italy	N:51.5 ± 12.3 C:40.1 ± 13.1	84/101	LB	HB	Sequencing
Alam[34]	2017	Bangladesh	N:39.1 ± 8.6 C:29.64 ± 7.03	75/99	LB	HB	TaqMan
Mazo[35]	2018	Brazil	N:24-76 C:19-78	127/255	LB	HB	TaqMan
Yang[36]	2018	China	N:70.95 ± 4.73 C:72.53 ± 5.67	158/301	US	PB	Real-time PCR
Costanzo[37]	2019	Italy	N:11.0 ± 2.8 C:9.6 ± 2.7	131/99	AMRI	PB	TaqMan
Hudert[38]	2019	Germany	N:14.11 ± 2.15 C:46.73 ± 16.03	177/93	LB	PB	TaqMan
Wu[39]	2020	China	N:57.7 ± 8.53 C:58.90 ± 5.53	NA	US	PB	Real-time PCR
Narayanasamy[40]	2020	India	N:43.15 ± 9.245 C:41.99 ± 12.752	NA	US	PB	PCR-RFLP
Lisboa[41]	2020	Brazil	N:46.3 - 63 C:43 - 61.5	77/208	LB	HB	Real-time PCR
Delik[42]	2020	Turkey	N:46.09 ± 10 C:44.69 ± 11.28	155/174	LB	HB	Real-time TaqMan
Akkiz[43]	2021	Turkey	N:47.04 ± 12.2 C:26.9 ± 8.6	NA	LB	HB	Real-time PCR
Zhang[44]	2021	China	N:38.2 ± 13.78 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, PCR-RFLP = 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.[16] 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.[17] It confirmed that PNPLA3 possesses triacylglycerol hydrolase and acylglycerol transacylase activities.[18] Its gene mutation may affect fat metabolism, including the fat synthesis and the fat hydrolysis.[19] The PNPLA3 gene is located on human chromosome 22. The most studied focus on PNPLA3 gene, rs738,409 [g/ C], encodes i148m.[20] The i148m mutation is assigned isoleucine (I) at position 148 and methionine (M) at position 148, which causes a guanine to cytosine substitution (C→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.[22] The G allele of PNPLA3 rs738,409 is found to increase the risk of steatosis, lobular inflammation, hepatocyte ballooning.[23] 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.[24] 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.

Table 2

The distribution of alleles and genotypes of PNPLA3 in NAFLD studies.

	Sample size		Genotype in cases			Genotype in controls			Case		Control			
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
Narayanasamy	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 definition of cases	Representativeness of the cases	Selection of controls	Definition of controls	Comparability of cases and controls on the basis of the design or analysis	Ascertainment of exposure	Same method of ascertainment for cases and controls	Nonresponse rate	Quality 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-Gentilucci 2016	☆	☆	☆		☆☆	☆	☆		7
Alam 2017	☆	☆		☆	☆	☆	☆	☆	7
Mazo 2018	☆	☆		☆	☆	☆	☆	☆	7
Yang 2018	☆	☆	☆	☆	☆	☆	☆	☆	8
Costanzo 2019	☆	☆		☆	☆☆	☆	☆		7
Hudert 2019	☆	☆	☆	☆	☆☆	☆	☆	☆	9
Wu 2020	☆	☆		☆	☆☆	☆	☆	☆	8
Narayanasamy 2020	☆	☆	☆		☆☆	☆	☆		7
Lisboa 2020	☆	☆	☆		☆☆	☆	☆	☆	8
Delik 2020	☆	☆	☆		☆☆	☆	☆		7
Akkiz 2021	☆	☆		☆	☆☆	☆	☆		7
Zhang 2021	☆	☆	☆		☆☆	☆	☆	☆	8

2.2. Inclusive criteria

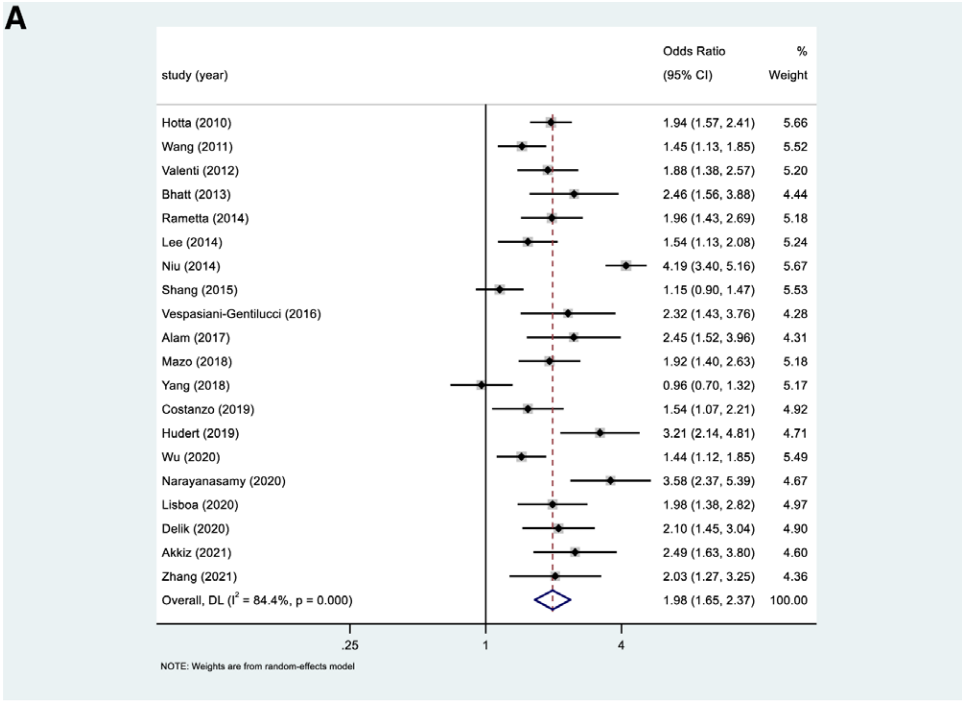
All the eligible studies were selected on the basis of the predefined inclusion criteria:

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 with corresponding 95% confidence interval (CI) in both cases and controls.

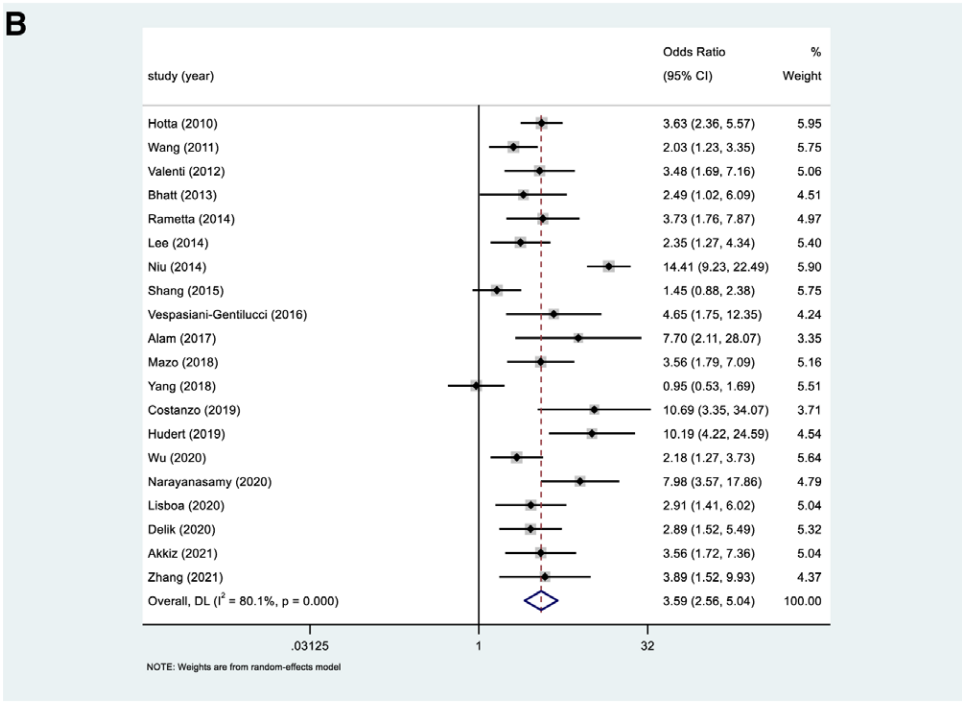
2.3. Exclusion criteria

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

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



allelic model（G vs C）



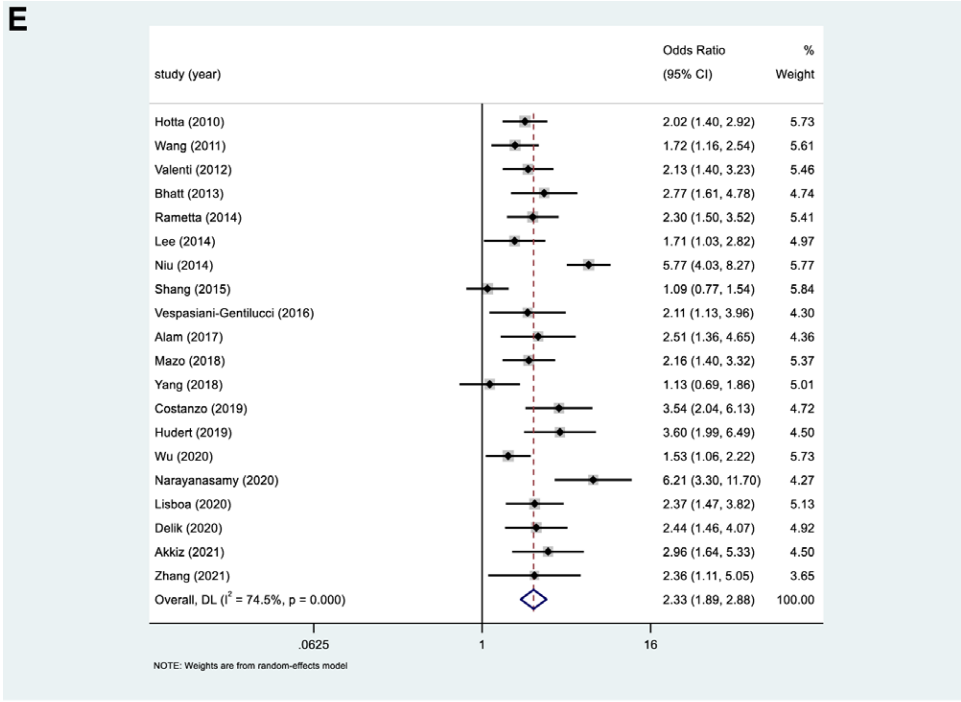
homozygote model（GG vs CC）

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



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).

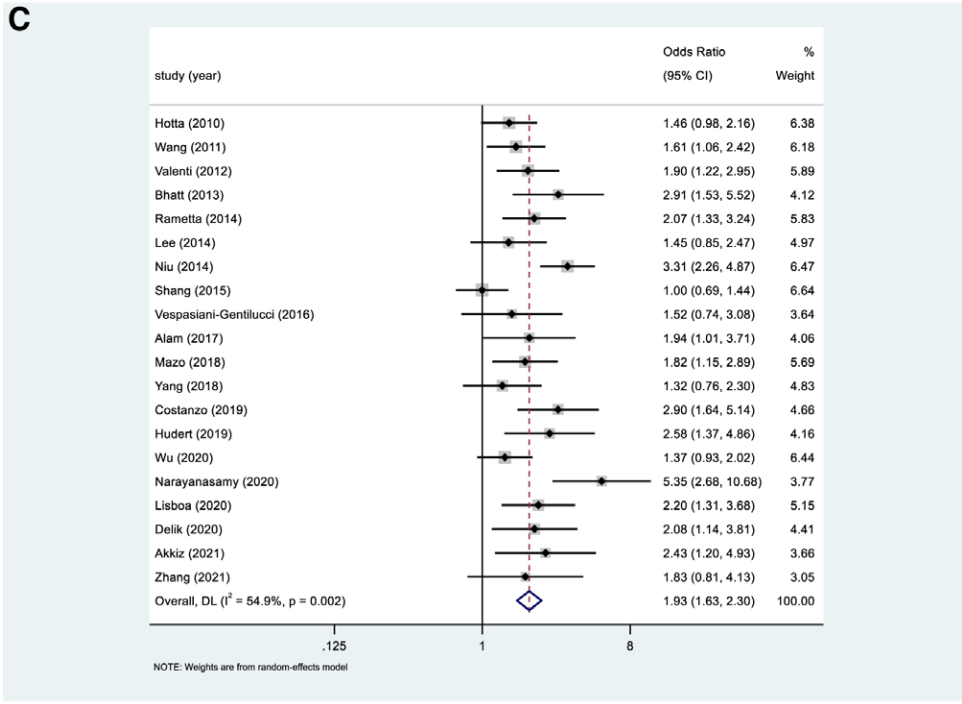
3. Results

3.1. Literature search

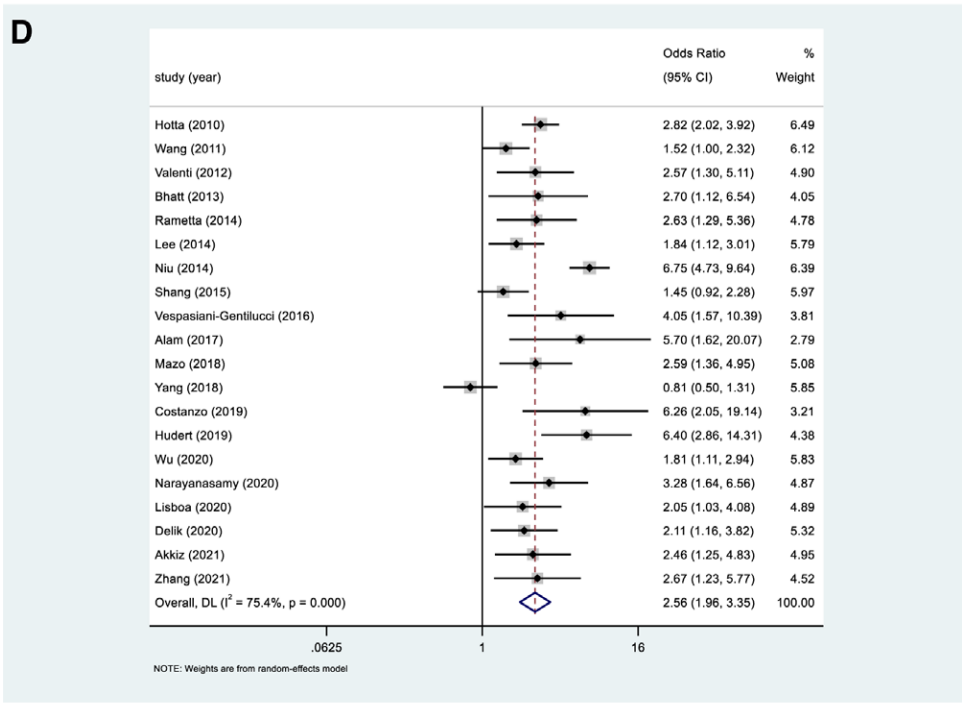
The flow diagram of study selection is presented in Figure 1. The search strategy initially identified 4171 potentially relevant articles, after the full-text reading, the repeated publications with the same data and review articles were excluded, leaving 2570 articles. After another round of full-text 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.



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, $P_{heterogeneity}$ = 0.000, Z = 7.346, P = .000), the homozygote comparison (OR = 3.59, 95% CI = 2.56–5.04, $P_{heterogeneity}$ = 0.000, Z = 7.416, P = .000), the heterozygote comparison (OR = 1.93, 95% CI = 1.63–2.30, $P_{heterogeneity}$ = 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) (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

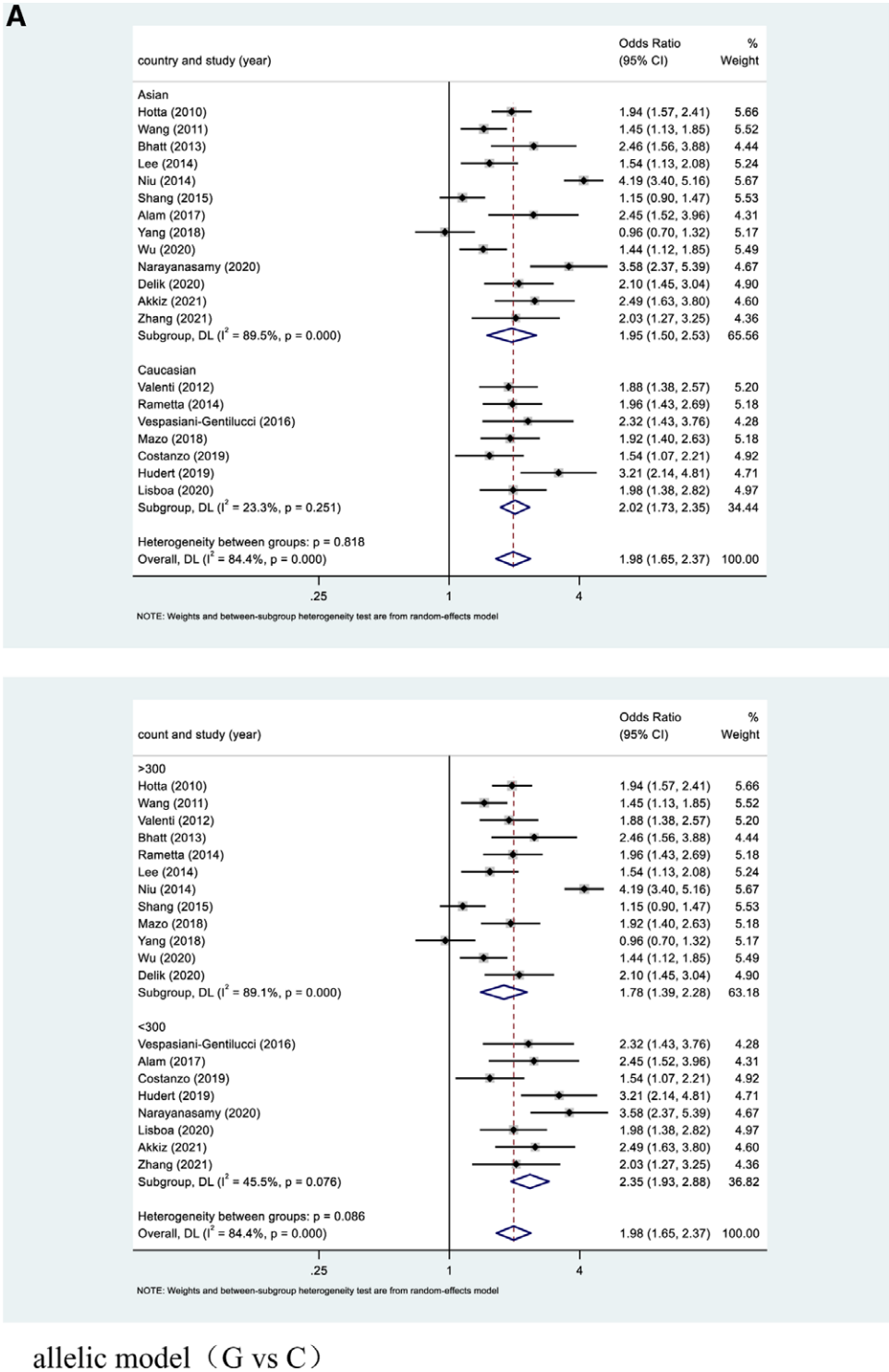


Figure 3. Subgroup analysis.

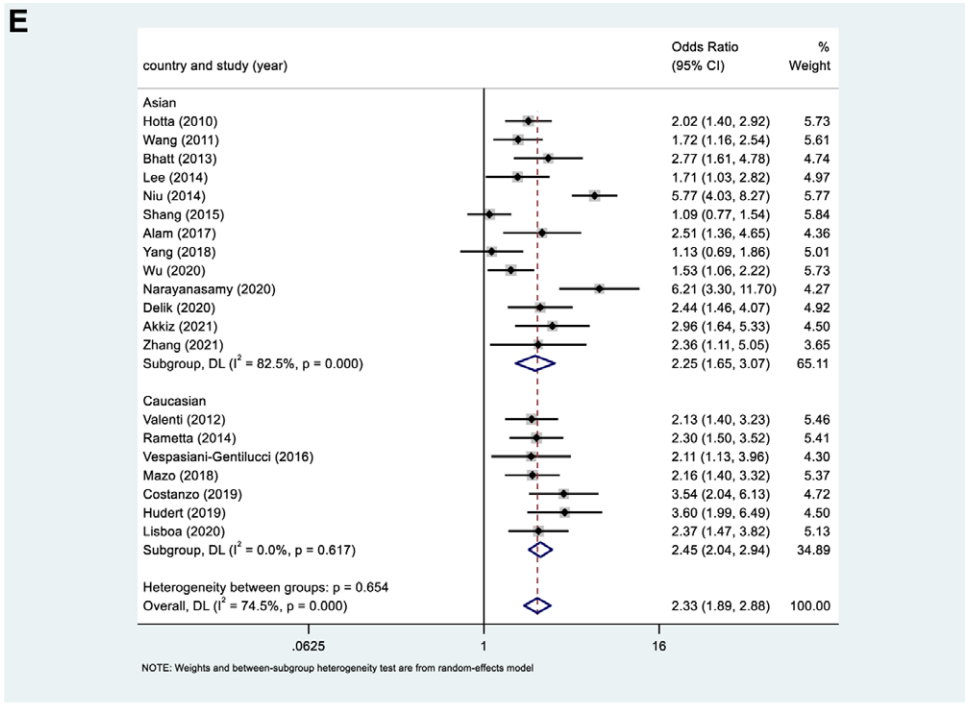
alanine aminotransferase and 4 articles mentioned aspartate aminotransferase. As shown in Figure 4, there is a great heterogeneity. However, there is no statistical difference.

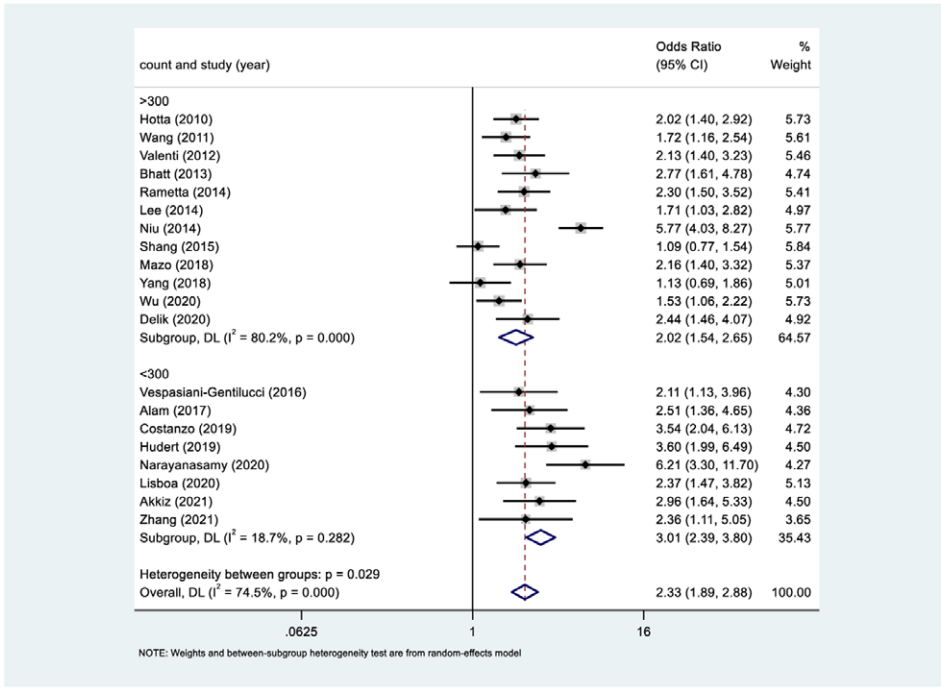
3.3.1. Sensitivity study. A sensitivity study was performed to estimate the effects of each individual study on the pooled OR. The sensitivity analysis indicates that none of the individual study alters the pooled results significantly. It confirms that our results are credible and generalizable (Fig. 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.[45] However, some other research shows that they are closely related.[46] Our study focuses on the relationship between PNPLA3 rs738,409 gene diversity and NAFLD.





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

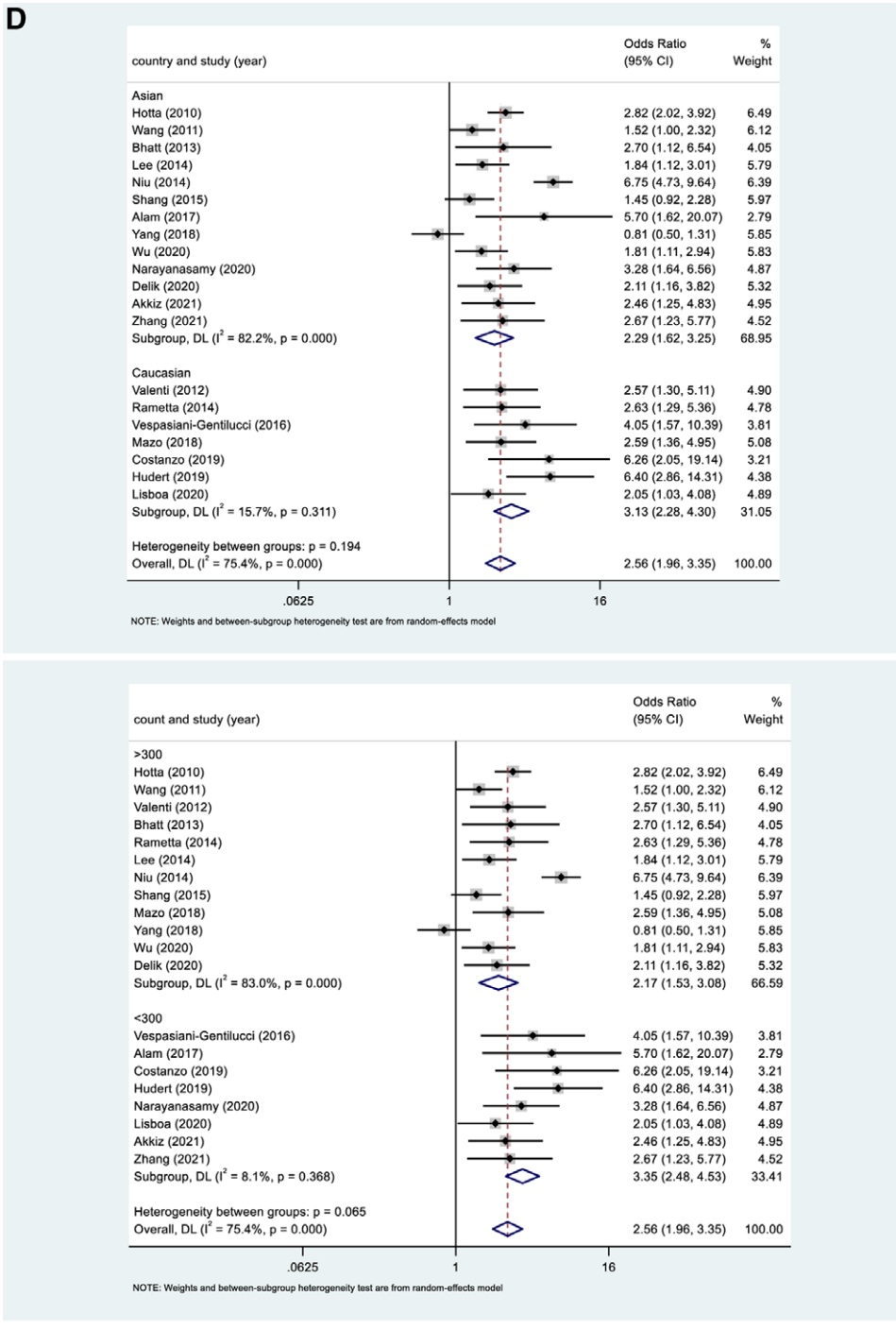


Figure 3. Continued

and monoacylglycerol).[47] 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.[48] 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.[49] 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.[50] 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;[51] 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.[52] The specific function

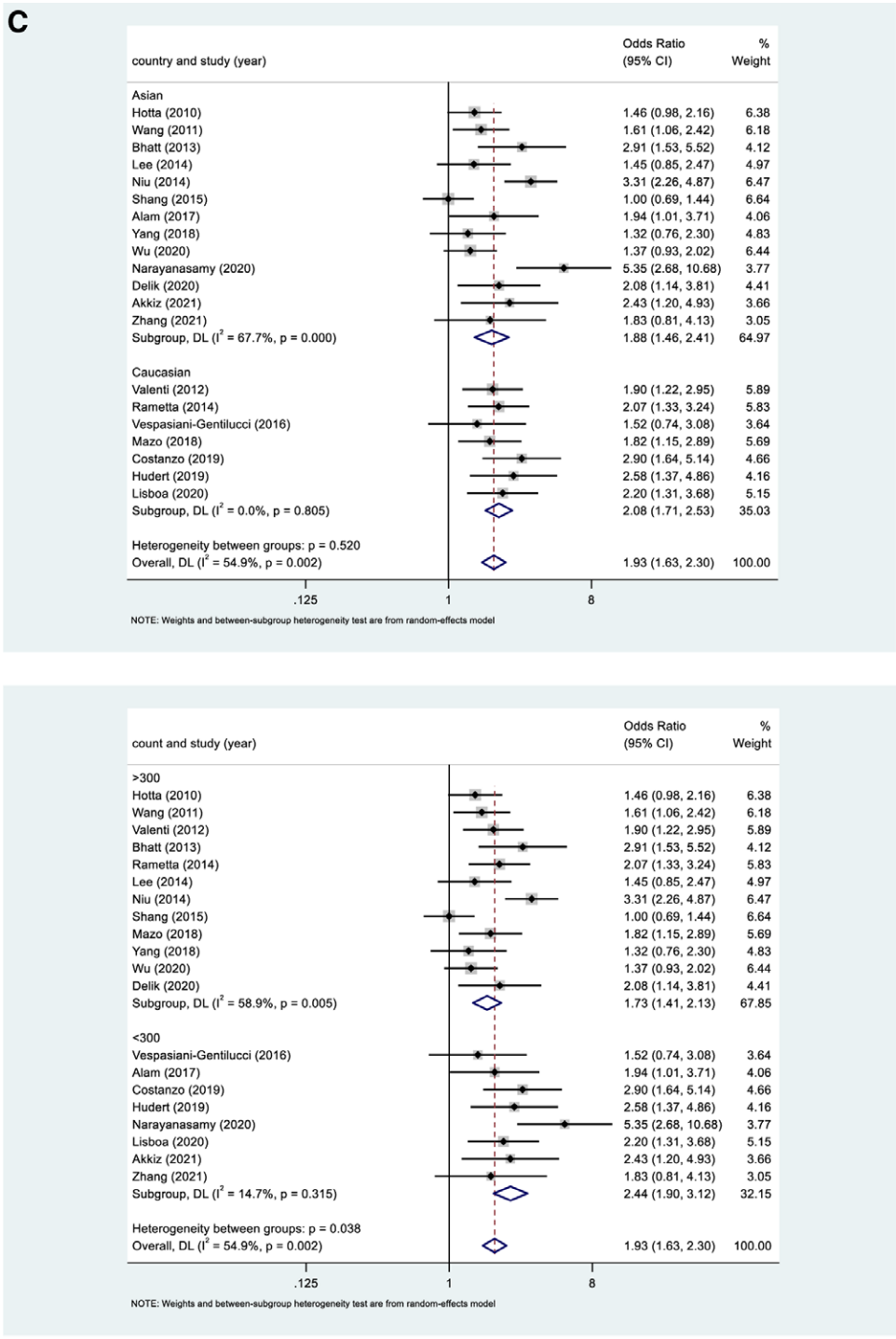


Figure 3. Continued

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.[53] 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

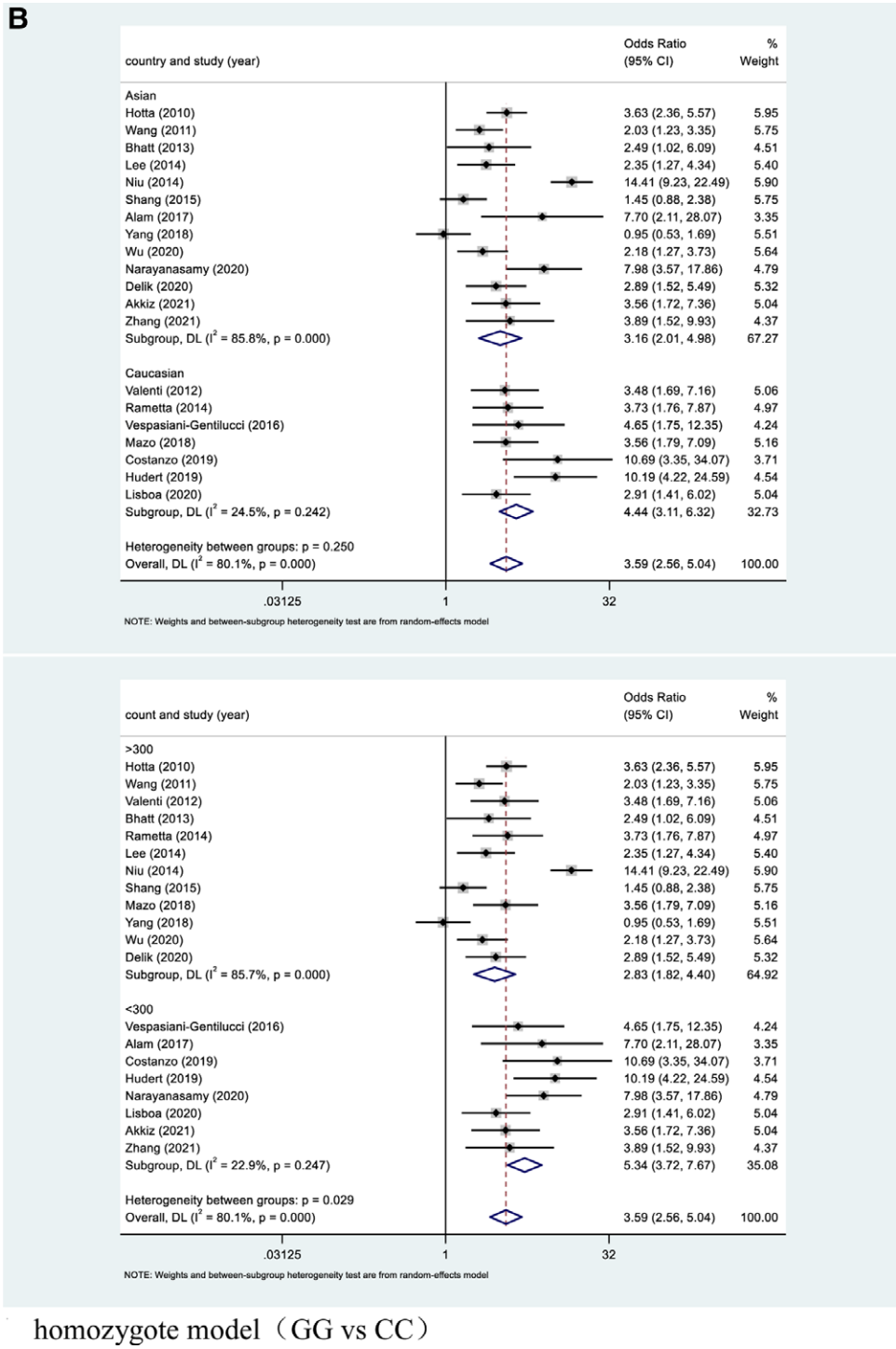


Figure 3. Continued

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

5. Conclusion

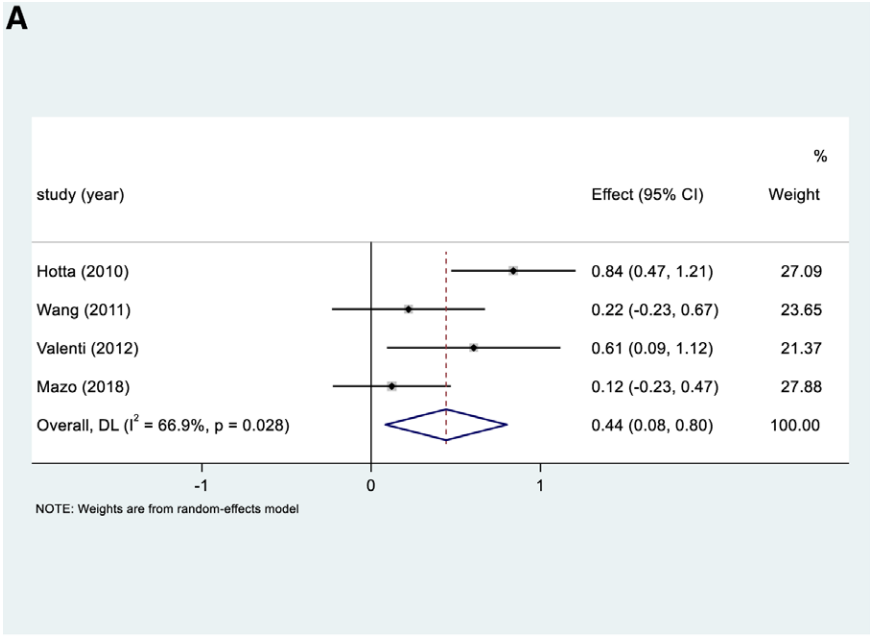
PNPLA3 rs738,409 may play a significant role in increasing risk of NAFLD.

Acknowledgment

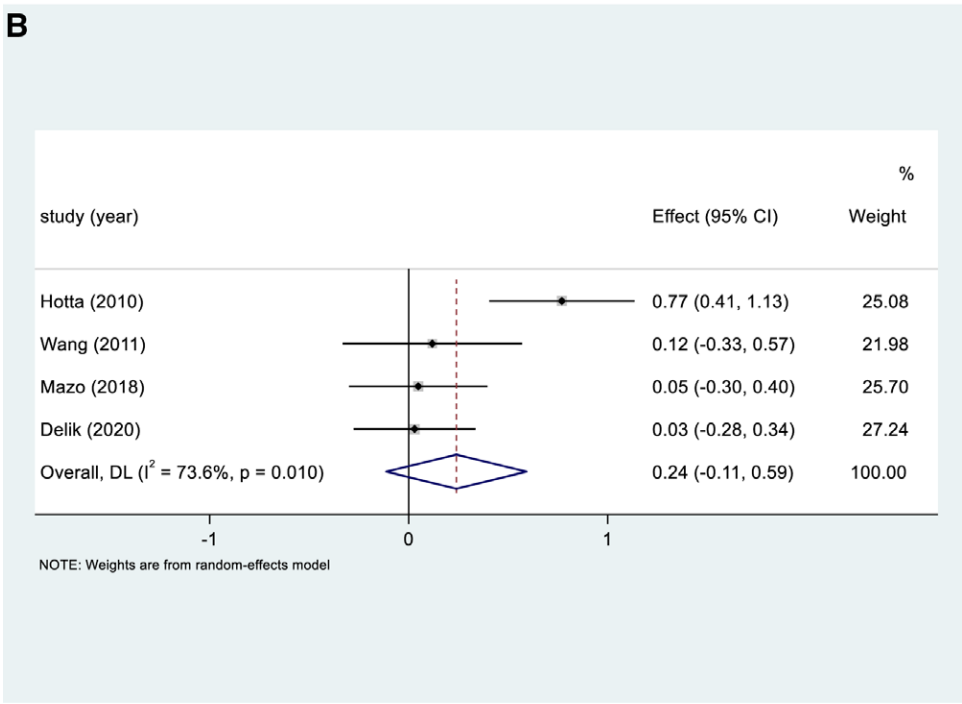
The authors thank Dr Bin Wang for assistance with data extraction.

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.

References

[1] Charlton MR, Burns JM, Pedersen RA, et al. Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. Gastroenterology. 2011;141:1249–53.
[2] Ye Q, Zou B, Yeo YH, et al. Global prevalence, incidence, and outcomes of non-obese or lean nonalcoholic fatty liver disease: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 2020;5:739–52.
[3] Estes C, Anstee QM, Arias-Loste MT, et al. Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016-2030. J Hepatol. 2018;69:896–904.
[4] ZhanZhan L, Xue J, Chen P, et al. Prevalence of nonalcoholic fatty liver disease in mainland of China: a meta-analysis of published studies. J Gastroenterol Hepatol. 2014;29:42–51.
[5] Arab JP, Arrese M, Trauner M. Recent insights into the pathogenesis of nonalcoholic fatty liver disease. Annu Rev Pathol. 2018;13:321–50.
[6] Wobser H, Dorn C, Weiss TS, et al. Lipid accumulation in hepatocytes induces fibrogenic activation of hepatic stellate cells. Cell Res. 2009;19:996–1005.

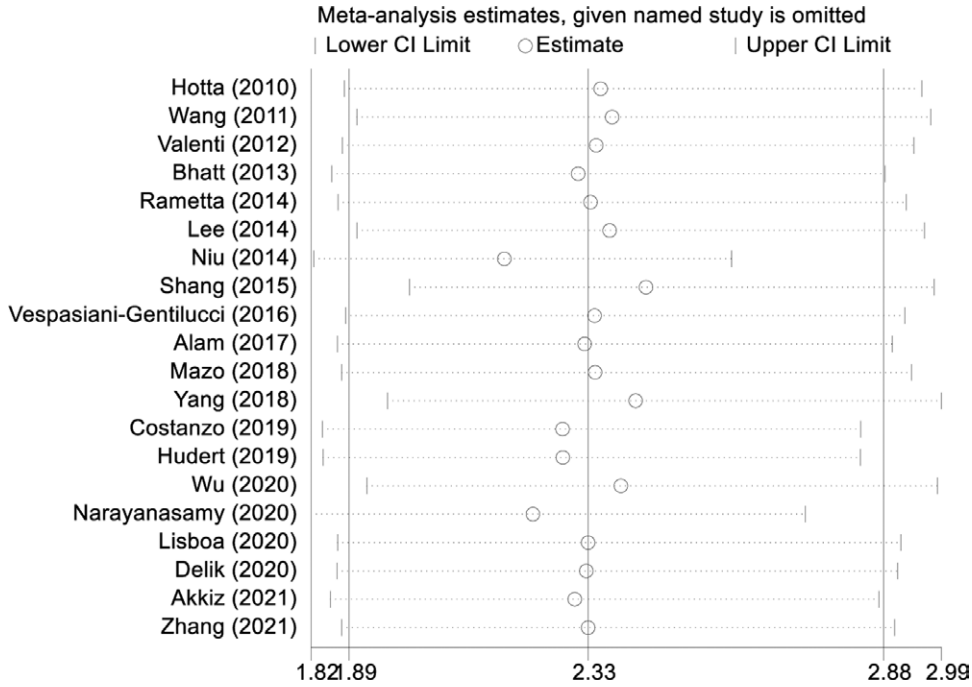


Figure 5. Sensitivity analysis of the dominant model.

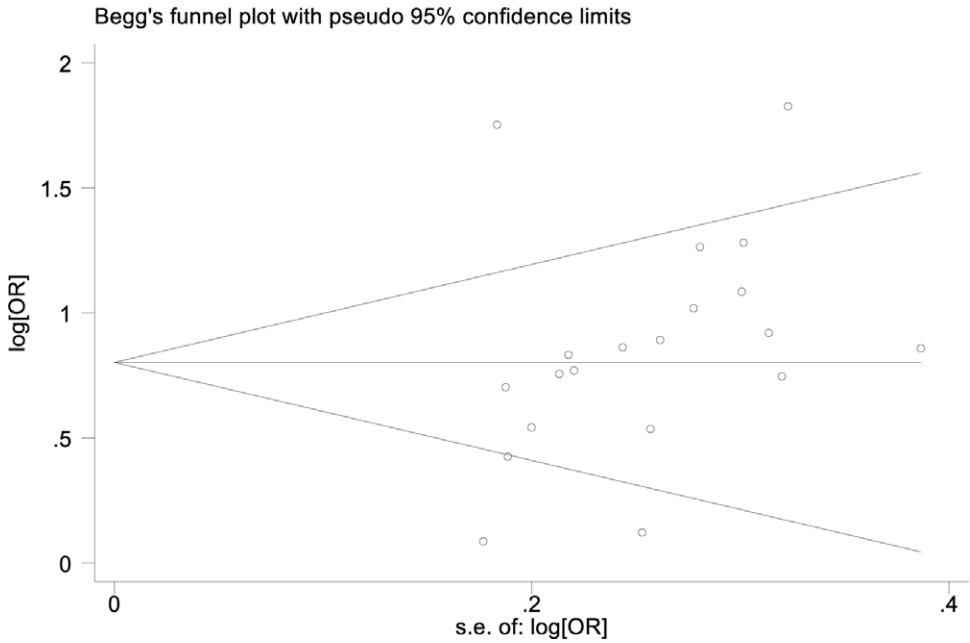


Figure 6. Begg funnel plot of dominant model.

[7] National Workshop on Fatty Liver and Alcoholic Liver Disease, Chinese Society of Hepatology, Chinese Medical Association, Fatty Liver Expert Committee, Chinese Medical Doctor Association. Guidelines of prevention and treatment for nonalcoholic fatty liver disease: a 2018 update. Zhonghua gan zang bing za zhi. 2018;26:195–203.
[8] Ganji SH, Kashyap ML, Kamanna VS. Niacin inhibits fat accumulation, oxidative stress, and inflammatory cytokine IL-8 in cultured hepatocytes: Impact on nonalcoholic fatty liver disease. Metabolism. 2015;64:982–90.
[9] Taegtmeyer H, Stanley WC. Too much or not enough of a good thing? Cardiac glucolipotoxicity versus lipoprotection. J Mol Cell Cardiol. 2011;50:2–5.
[10] Virtue S, Vidal-Puig A. Adipose tissue expandability, lipotoxicity and the metabolic syndrome. Endocrinol Nutr. 2013;60:39–43.
[11] Zhang L, Keung W, Samokhvalov V, et al. Role of fatty acid uptake and fatty acid beta-oxidation in mediating insulin resistance in heart and skeletal muscle. Biochim Biophys Acta. 2010;1801:1–22.
[12] Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of nonalcoholic fatty liver disease (NAFLD). Metabolism. 2016;65:1038–48.
[13] Friedman SL, Neuschwander-Tetri BA, Rinella M, et al. Mechanisms of NAFLD development and therapeutic strategies. Nat Med. 2018;24:908–22.
[14] Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology. 2012;55:2005–23.

[15] Zheng YC, Jia W. Research progress of exercise prescription for nonalcoholic fatty liver disease. Occup Health. 2020;36:569–72.
[16] Bruschi FV, Tardelli M, Claudel T, et al. PNPLA3 expression and its impact on the liver: current perspectives. Hepat Med. 2017;9:55–66.
[17] Basu Ray S. PNPLA3-I148M: a problem of plenty in nonalcoholic fatty liver disease. Adipocyte. 2019;8:201–8.
[18] Liu W, Anstee QM, Wang X, et al. Transcriptional regulation of PNPLA3 and its impact on susceptibility to nonalcoholic fatty liver Disease (NAFLD) in humans. Aging (Albany NY). 2016;9:26–40.
[19] Dongiovanni P, Donati B, Fares R, et al. PNPLA3 I148M polymorphism and progressive liver disease. World J Gastroenterol. 2013;19:6969–78.
[20] Carpino G, Pastori D, Baratta F, et al. PNPLA3 variant and portal/periportal histological pattern in patients with biopsy-proven nonalcoholic fatty liver disease: a possible role for oxidative stress. Sci Rep. 2017;7:15756.
[21] Valenti L, Maggioni P, Piperno A, et al. Patatin-like phospholipase domain containing-3 gene I148M polymorphism, steatosis, and liver damage in hereditary hemochromatosis. World J Gastroenterol. 2012;18:2813–20.
[22] Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet. 2008;40:1461–5.
[23] Speliotes EK, Butler JL, Palmer CD, et al. PNPLA3 variants specifically confer increased risk for histologic nonalcoholic fatty liver disease but not metabolic disease. Hepatology. 2010;52:904–12.
[24] Rotman Y, Koh C, Zmuda JM, et al. The association of genetic variability in patatin-like phospholipase domain-containing protein 3 (PNPLA3) with histological severity of nonalcoholic fatty liver disease. Hepatology. 2010;52:894–903.
[25] Hotta K, Yoneda M, Hyogo H, et al. Association of the rs738409 polymorphism in PNPLA3 with liver damage and the development of nonalcoholic fatty liver disease. BMC Med Genet. 2010;11:172.
[26] Wang CW, Lin HY, Shin SJ, et al. The PNPLA3 I148M polymorphism is associated with insulin resistance and nonalcoholic fatty liver disease in a normoglycaemic population. Liver Int. 2011;31:1326–31.
[27] Valenti L, Rametta R, Ruscica M, et al. The I148M PNPLA3 polymorphism influences serum adiponectin in patients with fatty liver and healthy controls. BMC Gastroenterol. 2012;12:111.
[28] Bhatt SP, Nigam P, Misra A, et al. Genetic variation in the patatin-like phospholipase domain-containing protein-3 (PNPLA-3) gene in Asian Indians with nonalcoholic fatty liver disease. Metab Syndr Relat Disord. 2013;11:329–35.
[29] Rametta R, Ruscica M, Dongiovanni P, et al. Hepatic steatosis and PNPLA3 I148M variant are associated with serum Fetuin-A independently of insulin resistance. Eur J Clin Invest. 2014;44:627–33.
[30] Lee SS, Byoun YS, Jeong SH, et al. Role of the PNPLA3 I148M polymorphism in nonalcoholic fatty liver disease and fibrosis in Korea. Dig Dis Sci. 2014;59:2967–74.
[31] Niu TH, Jiang M, Xin YN, et al. Lack of association between apolipoprotein C3 gene polymorphisms and risk of nonalcoholic fatty liver disease in a Chinese Han population. World J Gastroenterol. 2014;20:3655–62.
[32] Shang XR, Song JY, Liu FH, et al. GWAS-identified common variants with nonalcoholic fatty liver disease in Chinese children. J Pediatr Gastroenterol Nutr. 2015;60:669–74.
[33] Vespasiani-Gentilucci U, Gallo P, Porcari A, et al. The PNPLA3 rs738409 C > G polymorphism is associated with the risk of progression to cirrhosis in NAFLD patients. Scand J Gastroenterol. 2016;51:967–73.
[34] Alam S, Islam MS, Islam S, et al. Association of single nucleotide polymorphism at PNPLA3 with fatty liver, steatohepatitis, and cirrhosis of liver. Indian J Gastroenterol. 2017;36:366–72.
[35] Mazo DF, Malta FM, Stefano JT, et al. Validation of PNPLA3 polymorphisms as risk factor for NAFLD and liver fibrosis in an admixed population. Ann Hepatol. 2019;18:466–71.
[36] Yang H, Chen G, Song C, et al. A novel index including SNPs for the screening of nonalcoholic fatty liver disease among elder Chinese: a population-based study. Medicine (Baltim). 2018;97:e0272e0272.
[37] Di Costanzo A, Pacifico L, D'Erasmo L, et al. Nonalcoholic Fatty Liver Disease (NAFLD), but not its susceptibility gene variants, influences the decrease of kidney function in overweight/obese children. Int J Mol Sci . 2019;20:4444.
[38] Hudert CA, Selinski S, Rudolph B, et al. Genetic determinants of steatosis and fibrosis progression in paediatric nonalcoholic fatty liver disease. Liver Int. 2019;39:540–56.
[39] Wu JT, Liu SS, Xie XJ, et al. Independent and joint correlation of PNPLA3 I148M and TM6SF2 E167K variants with the risk of coronary heart disease in patients with nonalcoholic fatty liver disease. Lipids Health Dis. 2020;19:29.
[40] Narayanasamy K, Karthick R, Panneerselvam P, et al. Association of metabolic syndrome and patatin-like phospholipase 3 - rs738409 gene variant in nonalcoholic fatty liver disease among a Chennai-based south Indian population. J Gene Med. 2020;22:e3160.
[41] Lisboa QC, Nardelli MJ, Pereira PA, et al. PNPLA3 and TM6SF2 polymorphisms in Brazilian patients with nonalcoholic fatty liver disease. World J Hepatol. 2020;12:792–806.
[42] Delik A, Akkız H, Dinçer S. The effect of PNPLA3 polymorphism as gain in function mutation in the pathogenesis of nonalcoholic fatty liver disease. Indian J Gastroenterol. 2020;39:84–91.
[43] Akkiz H, Taskin E, Karaogullarindan U, et al. The influence of RS738409 I148M polymorphism of patatin-like phospholipase domain containing 3 gene on the susceptibility of nonalcoholic fatty liver disease. Medicine (Baltim). 2021;100:e25893.
[44] Zhang RN, Shen F, Pan Q, et al. PPARGC1A rs8192678 G>A polymorphism affects the severity of hepatic histological features and nonalcoholic steatohepatitis in patients with nonalcoholic fatty liver disease. World J Gastroenterol. 2021;27:3863–76.
[45] Li X, Zhao Q, Wu K, et al. I148M variant of PNPLA3 confer increased risk for nonalcoholic fatty liver disease not only in European population, but also in Chinese population. Hepatology. 2011;54:2275.
[46] Li Y, Xing C, Cohen JC, et al. Genetic variant in PNPLA3 is associated with nonalcoholic fatty liver disease in China. Hepatology. 2012;55:327–8.
[47] Li Q, Qu HQ, Rentfro AR, et al. PNPLA3 polymorphisms and liver aminotransferase levels in a Mexican American population. Clin Invest Med. 2012;35:E237–45.
[48] Qu HQ, Li Q, Grove ML, et al. Population-based risk factors for elevated alanine aminotransferase in a South Texas Mexican-American population. Arch Med Res. 2012;43:482–8.
[49] Chan DC, Watts GF, Gan S, et al. Nonalcoholic fatty liver disease as the transducer of hepatic oversecretion of very-low-density lipoprotein-apolipoprotein B-100 in obesity. Arterioscler Thromb Vasc Biol. 2010;30:1043–50.
[50] Pirazzi C, Adiels M, Burza MA, et al. Patatin-like phospholipase domain-containing 3 (PNPLA3) I148M (rs738,409) affects hepatic VLDL secretion in humans and in vitro. J Hepatol. 2012;57:1276–82.
[51] Green CJ, Johnson D, Amin HD, et al. Characterization of lipid metabolism in a novel immortalized human hepatocyte cell line. Am J Physiol Endocrinol Metab. 2015;309:E511–22.
[52] Hao L, Ito K, Huang KH, et al. Shifts in dietary carbohydrate-lipid exposure regulate expression of the nonalcoholic fatty liver disease-associated gene PNPLA3/adiponutrin in mouse liver and HepG2 human liver cells. Metabolism. 2014;63:1352–62.
[53] Unalp-Arida A, Ruhl CE. Patatin-like phospholipase domain-containing protein 3 I148M and liver fat and fibrosis scores predict liver disease mortality in the U.S. population. Hepatology. 2020;71:820–34.