

Original Article

Association between patatin-like phospholipase domain-containing 3 gene (PNPLA3) polymorphism and overweight/obesity: a meta-analysis

Qin Wu¹, Cong Wang², Jiping Li²

¹Infectious Diseases Center, West China Hospital, Sichuan University, Chengdu, Sichuan, China; ²Department of Nursing, West China Hospital, Sichuan University, Chengdu 610041, Sichuan, China

Received December 24, 2015; Accepted April 10, 2016; Epub July 15, 2016; Published July 30, 2016

Abstract: Background: The patatin-like phospholipase domain-containing 3 (PNPLA3, rs738409) gene polymorphism has been implicated in susceptibility to overweight and obesity, but study results are still controversial. Objective: The present meta-analysis was performed to evaluate the relationship between PNPLA3 polymorphism and overweight/obesity among adults, children or adolescents. Methods: A literature search was conducted in PubMed, Medline, Embase, EBSCO and Web of Science databases up to December 2015. Statistical analysis of qualified studies was performed with Stata 12.0 software. Results: Ten studies comprising 14,220 participants (7,120 cases and 7,100 controls) were included in the analysis. A total of 6,963 patients had the rs738409 GG+CG genotype, whereas 7,257 patients had the CC genotype. No evidence of an association between the rs738409 polymorphism and overweight/obesity risk was observed in incorporated patients (OR = 0.929, 95% CI: 0.863-1.000; P = 0.051). Ethnicity stratification analyses revealed that the G allele (GG+CG) did not show an unfavorable effect compared to CC genotype in Caucasians (OR = 0.960, 95% CI: 0.885-1.042; P = 0.327) and Asians (OR = 0.707, 95% CI: 0.575-0.869; P = 0.001). Also, no significant association was found between the PNPLA3 gene polymorphism and the risk of overweight/obesity when the population was stratified by age (children or adolescents, adults) and non-alcoholic fatty liver disease (NAFLD). Conclusion: Our meta-analysis revealed that the PNPLA3 gene rs738409 polymorphism was not associated with an enhanced risk of overweight/obesity among Caucasians, neither adults nor children or adolescents. This conclusion accredits confirmation by more case-control and cohort studies.

Keywords: Obesity, overweight, PNPLA3, gene polymorphism, meta-analysis

Introduction

Currently, an estimation of at least 300 million people worldwide are obese [1], which poses a global health problem among children and adolescents, caused a broad range of disorders such as diabetes mellitus, hypertension, cardiovascular disease and cancer [2]. Pathogenesis of overweight or obesity is multifactorial and involves the environment, genetic predisposition, and human behavior [3, 4]. The genetic pathways indicate a significant involvement in the occurrence and development of obesity [5, 6]. However, the genetic pathways underlying obesity still remain elusive.

One variant has garnered much research attention in the past few years. Early in 2008, Romeo and colleagues [7] discovered a novel sin-

gle nucleotide polymorphism (SNP) in the patatin-like phospholipase domain-containing 3 (PNPLA3, rs738409) localized on human chromosome 22, which was designated the PNPLA3 protein (also known as adiponutrin) [8]. The substitution from cytosine to guanine in rs738409 C>G caused an amino acid change from isoleucine (I) to methionine (M) at residue 148 (I148M) of the protein, the G allele frequently to be adverse allele, was reported to be associated with an increased liver fat content. In addition, the variant polymorphism exhibits lipase activity against triglycerides and acylglycerol transacetylase activity, and its expression delivers highly responsive in energy mobilization and the storage of lipid droplets [9], which regulates weight gain and obesity. Subsequently, studies have identified an association of this

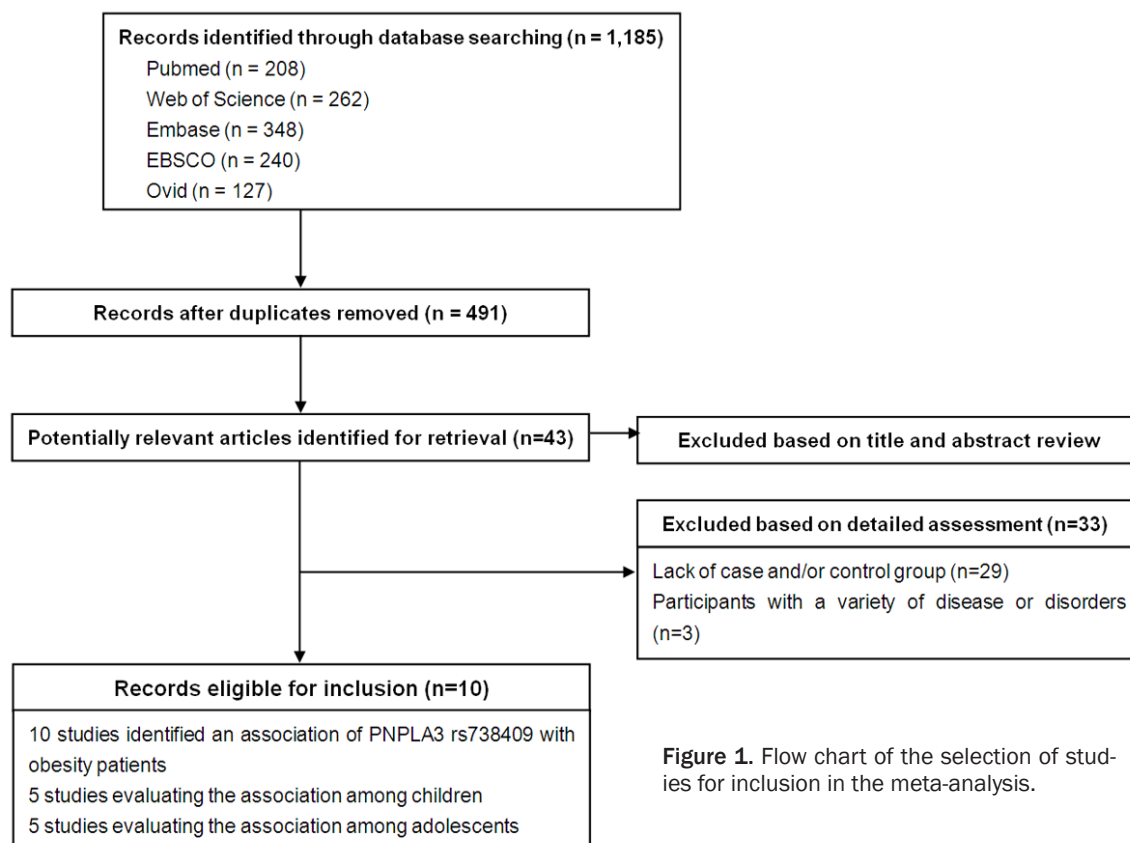


Figure 1. Flow chart of the selection of studies for inclusion in the meta-analysis.

SNP with overweight and obese patients [10-12]. Therefore, this SNP seems not only to be a risk factor in the development of liver fat content but also overweight/obesity. However, the association between PNPLA3 and overweight/obesity is still less; indeed, the influence of PNPLA3 variation on obesity is limited by sample size.

Meta-analyses of genetic association studies have the benefit to overcome the limitations and evaluate the strength of an association [13]. Therefore, we performed a meta-analysis to analyze the influence of this polymorphism on overweight/obese patients, and to estimate the strength of this association.

Materials and methods

Search strategy

The initial search was done on December 8, 2015 and no language or time restrictions were applied. Relevant studies were identified by a PubMed, Medline, Embase, EBSCO and Web of Science literature search with the fol-

lowing terms: (obesity or adipose tissue or overweight or weight or body mass index or BMI) and (adiponutrin, human or PNPLA3 or patatin-like phospholipase domain containing 3 or rs738409). Additionally, references of eligible studies were also screened for any potential studies.

Study selection

Inclusion and exclusion criteria were established prior to initiation of the literature search.

Inclusion criteria were as follows: (1) case-control and cohort studies; (2) studies evaluating the relationship between rs738409 polymorphism and the risk of overweight or obesity; (3) studies including overweight or obesity as the cases versus groups of controls; (4) studies that included cases or controls with different phenotypes had to provide separate genotype information; (5) studies have been published as a whole-length article.

Exclusion criteria were as follows: (1) studies that do not meet the inclusion criteria; (2) dis-

Association between PNPLA3 and overweight/obesity

Table 1. Characteristics of the 10 studies included in meta-analysis

Subgroup ID	First author	Year	Ethnicity (region)	Age groups	Genotype method	Criteria for selection of cases	Total	Males	Population types	HWE
1	Nikolaj Thure Krarup	2012	Caucasian (Denmark)	Adults	KASPar SNP Genotyping	BMI $\geq$ 30 kg/m ² or a waist-to-hip ratio $\geq$ 0.9 (men) and 0.85 (women)	4328	2285	Healthy Population	T
2	Elena Larrieta-Carrasco	2013	Mexican Mestizo (Mexico)	Children	TaqMan SNP assays	BMI percentile $\geq$ 85 th	1037	493	Healthy Population	T
3	S. Lallukka	2013	Caucasian (Finland)	Adults	Real-time PCR	BMI SD values	82	19	NAFLD Population	F
4	A. Viitasalo	2014	Caucasian (Finland)	Children	TaqMan SNP assays and Real-time PCR	The age- and sex- specific BMI cut-offs of the IOTF	481	254	Healthy Population	T
5	Elena Larrieta-Carrasco	2014	Mexican Mestizo (Mexico)	Adults	TaqMan SNP assays	BMI $\geq$ 25 kg/m ²	529	144	Healthy Population	T
6	Ivana A. Stojkovic	2014	Caucasian (Sweden)	Adults	TaqMan SNP assays	BMI>25 kg/m ²	4824	-	Healthy Population	T
7	H. Mangge	2015	Caucasian (Austria)	Children/ Adults	TaqMan SNP assays	BMI percentile $\geq$ 85 th (Youth), BMI $\geq$ 25 kg/m ² (Adults:)	510	309	Healthy Population	F
8	Kenichi Nishioji	2015	Asian (Japan)	Adults	TaqMan SNP assays	BMI $\geq$ 25 kg/m ²	824	548	Healthy Population	T
9									NAFLD Population	
10	Kentaro Oniki	2015	Asian (Japan)	Adults	Real-time PCR	BMI $\geq$ 25 kg/m ²	740	478	Healthy Population	T
11									NAFLD Population	
12	Xiao-Rui Shang	2015	Asian (China)	Children/ Adolescents	MassARRAY System	The age- and sex- specific BMI cut-offs of the IOTF and BMI percentile $\geq$ 95 th	1027	574	Healthy Population	T
13									NAFLD Population	

Note: BMI, Body Mass Index; SD, Standard Deviation; NAFLD, Non Alcoholic Fatty Liver Disease; IOTF, International Obesity Task Force.

Association between PNPLA3 rs738409 and overweight/obesity

Table 2. Distributions of PNPLA3 rs738409 genotypes of eligible studies included in the meta-analysis

Subgroup ID	First author	Population types	Sample size		Genotype in cases			Genotype in controls		
			Case	Control	CC	CG	GG	CC	CG	GG
1	Nikolaj Thure Krarup	Healthy Population	2637	1691	1597	912	128	1010	593	88
2	Elena Larrieta-Carrasco	Healthy Population	486	551	80	231	175	84	248	219
3	S. Lallukka	NAFLD Population	41	41	29	-	12	26	-	15
4	A. Viitasalo	Healthy Population	58	423	36	22		247	176	
5	Elena Larrieta-Carrasco	Healthy Population	334	195	22	143	169	22	71	102
6	Ivana A. Stojkovic	Healthy Population	2478	2346	1561	824	93	1465	780	101
7	H. Mangge	Healthy Population	288	209	173	96	19	117	78	14
8	Kenichi Nishioji	Healthy Population	156	396	55	74	27	115	210	71
9		NAFLD Population	212	60	47	109	56	7	33	20
10	Kentaro Oniki	Healthy Population	70	402	27	34	9	116	215	71
11		NAFLD Population	64	55	14	35	15	9	34	12
12	Xiao-Rui Shang	Healthy Population	175	690	79	81	15	259	337	94
13		NAFLD Population	121	41	46	55	20	14	19	8

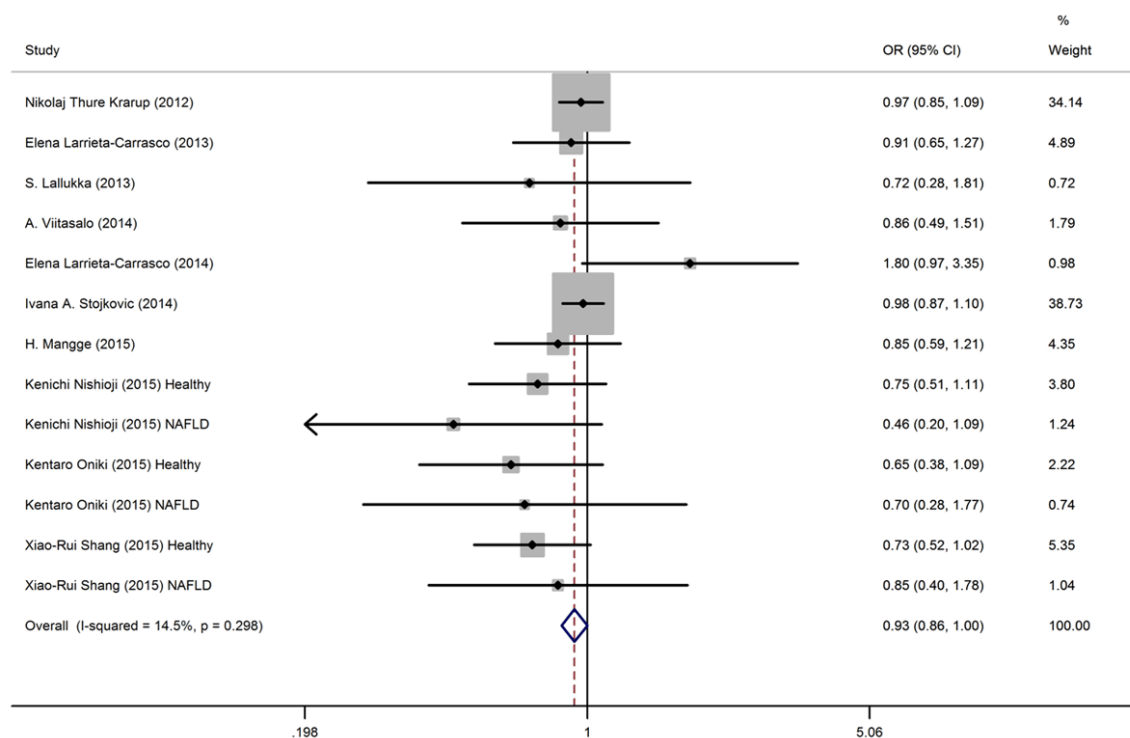


Figure 2. Forest plot for overall studies evaluating the association between overweight/obesity and the PNPLA3 rs738409 polymorphism.

eases affect body weight: cancer, diabetes, cardiovascular disease, polycystic ovary syndrome and other endocrine dysfunctions; (3) examining a highly specific population (e.g. pregnant women); (4) studies of posters, abstracts, reviews, letters, editorials, or clinical cases.

Data extraction and quality assessment

Data were systematically extracted by two independent investigators (CW, QW) using a standard protocol. When duplicate studies existed, only the most extensive data were taken into account. When several subgroups existed in a

Association between PNPLA3 and overweight/obesity

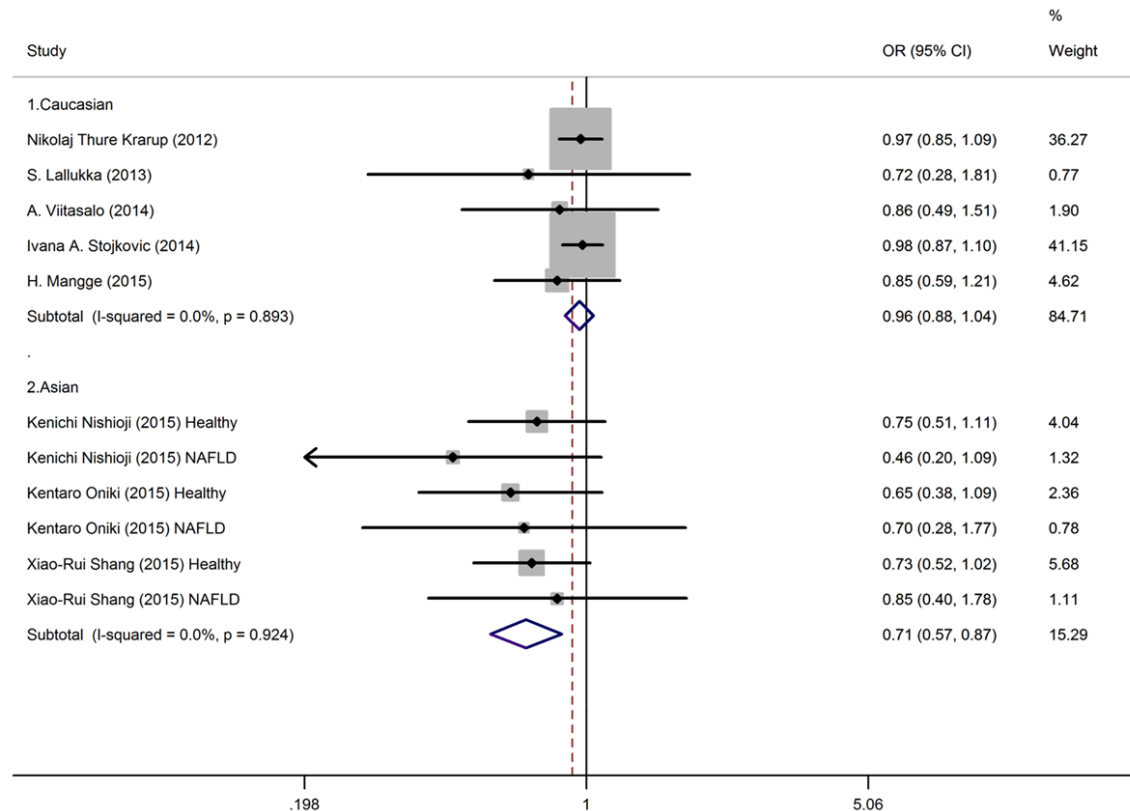


Figure 3. Forest plot of the association between overweight/obesity and the PNPLA3 rs738409 polymorphism stratified by ethnicity (Caucasian and Asian).

research, and some of them were unsatisfactory, only eligible subgroups were incorporated. When the data were not explicitly reported, we contacted corresponding authors to request the data. The studies were assessed using the standardized data collection forms modified from the Newcastle-Ottawa scale (NOS) (maximum score of 9) [14]. Discrepancies were resolved by consulting with a third investigator (JPL).

Statistical analysis

The association of rs738409 polymorphism with the risk of overweight/obesity was obtained by pooling odds ratio (OR) and 95% confidence intervals (CI). The significance of the pooled OR and 95% CI were calculated by the Z-test, and a *P*-value <0.05 was considered statistically significant. We assessed study heterogeneity basing on the Cochran's Q statistic and *I*² statistic [15]. Heterogeneity was considered significant when a Q statistic *P* < 0.1 or *I*² > 50%. Therefore, the fixed effects model (Mantel and Haenszel method [16]) was selected for homogeneous outcomes (*P* ≥ 0.1

or *I*² < 50%) and the random effects model (DerSimonian and Laird method [17]) was applied for heterogeneous outcomes (*P* < 0.1 or *I*² ≥ 50%). The potential outliers of the heterogeneity were detected by Galbraith plot [18]. This graphical method enabled us to check those studies which could have biased the combined estimate. In addition, the sensitivity analysis was conducted to evaluate the stability of results and to precisely investigate the heterogeneity. By the way, publication bias was observed by Begg's funnel plot and the Egger's linear regression test [18].

For rs738409, a recessive model was applied (GG+CG versus CC). Separate analyses were performed to reduce the potential heterogeneity. Therefore, summarized ORs were stratified by ethnicity (Caucasian, Asian and Mexican Mestizo), age (children or adolescents, adults), and non-alcoholic fatty liver disease (NAFLD). Statistical analysis was performed with Stata software (v. 12.0; Stata, College Station, TX), and a two tailed *P* value less than 0.05 was considered to be statistically significant.

Association between PNPLA3 and overweight/obesity

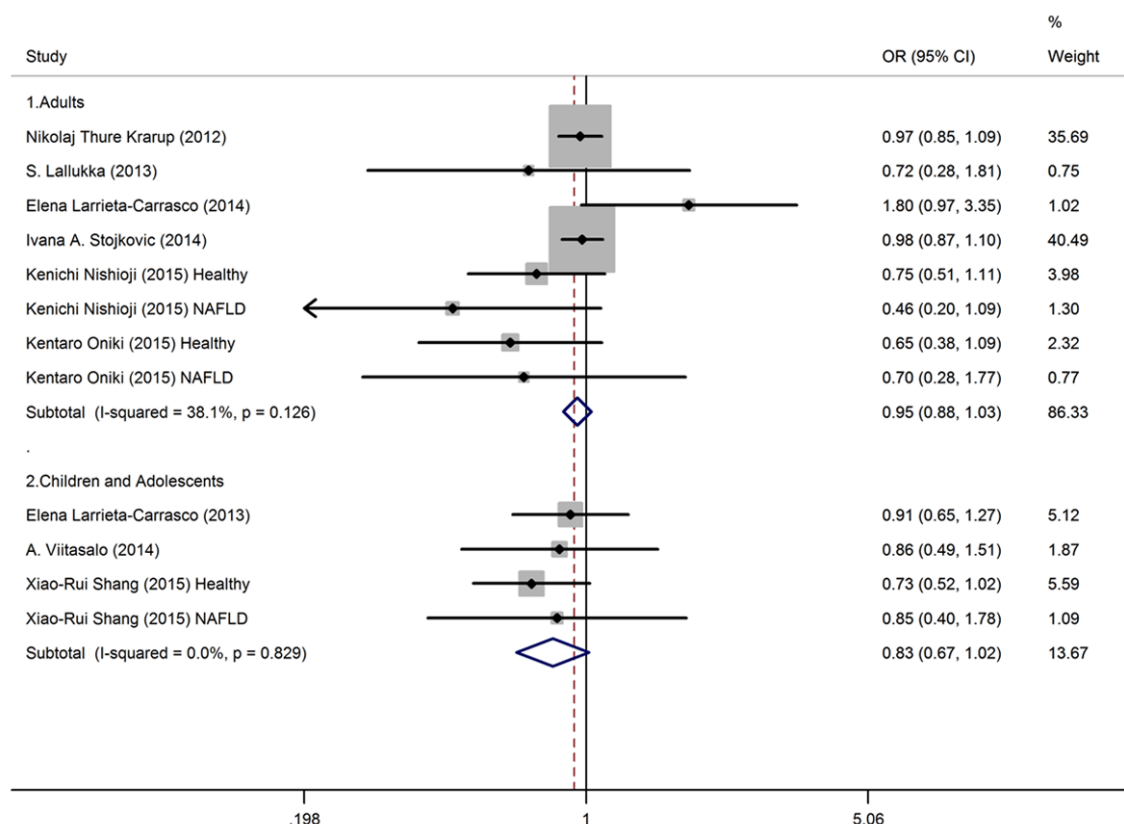


Figure 4. Forest plot of the association between overweight/obesity and the PNPLA3 rs738409 polymorphism stratified by age (children or adolescents, adults).

Results

Search results

The initial search yielded 1,185 entries for possible inclusion in our meta-analysis. **Figure 1** presents a flow chart of retrieved and excluded studies. Six hundred and ninety-four articles were excluded because of duplication. After screening the titles and abstracts, 448 articles were excluded because they were inconsistent with the inclusion criteria. This step left 43 references that were considered to have latent value, and the full texts were evaluated carefully. Among these, 33 studies were excluded because they lacked a case and/or control group. Three additional studies were excluded for participants with a variety of diseases such as diabetes. One more study was excluded because we were unable to compare rs738409 polymorphism and the risk of overweight/obesity. Finally, the article by Nishioji et al. [19], Oniki et al. [20], and Shang et al. [21] were considered two separate studies respectively, as the authors reported information from two dif-

ferent populations (with or without NAFLD). Thus, a total of 10 studies including 13 subgroups were included in this meta-analysis.

The characteristics of included studies are shown in **Table 1**, and the distribution of the rs738409 genotypes and alleles is presented in **Table 2**. According to quality assessment, these studies were relatively well-designed, and the quality assessments were at a high level. These studies (published from 2012 to 2015) comprising 14,220 participants (7,120 cases and 7,100 controls) were selected for the present study. Of all of the studies included, 5 studies involved Caucasians, 3 studies investigated Asians, and 2 studies researched Mexican Mestizo. In addition, 4 studies enrolled children or both children and adolescents, and 7 investigated adult patients. Three subgroups patients carried NAFLD.

Meta-analyses results

Ten studies stratifying into 13 subgroups were included in the analysis [10, 19-27]. A total of

Association between PNPLA3 and overweight/obesity

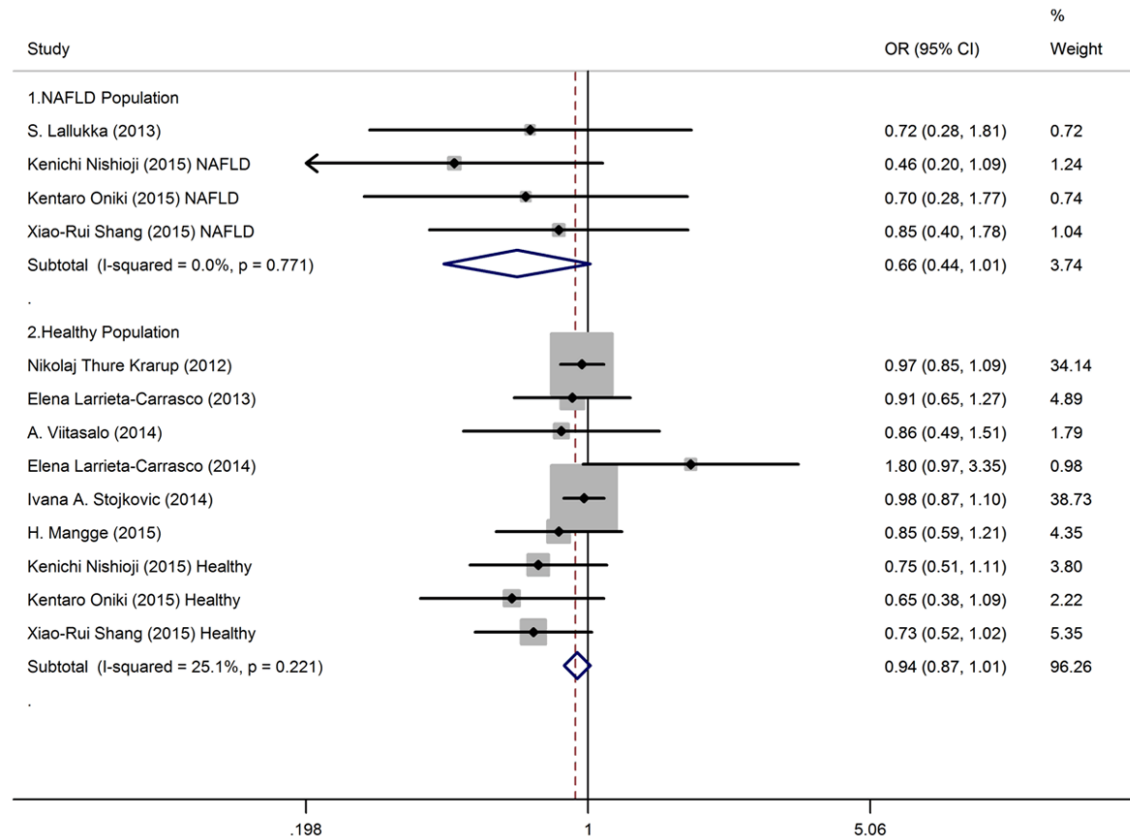


Figure 5. Forest plot of the association between overweight/obesity and the PNPLA3 rs738409 polymorphism stratified by non-alcoholic fatty liver disease.

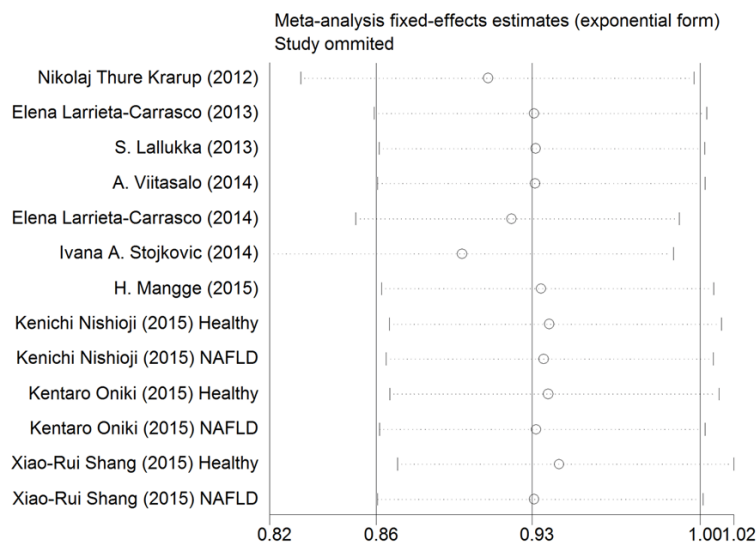


Figure 6. Sensitivity analysis showing the odds ratio and its 95% CI after sequentially omitting each study.

6,963 patients had the rs738409 GG+CG genotype, whereas 7,257 patients had the CC genotype.

otype. For PNPLA3 rs738409, the between-study heterogeneity was not significant when all 10 studies (13 subgroups) were pooled for analysis ($I^2 = 26.9\%$, $P = 0.173$); thus, we selected the fixed effect model. Mantel and Haenszel method was used.

No evidence of an association between the rs738409 polymorphism and obesity risk was observed in incorporated patients (OR = 0.929, 95% CI: 0.863-1.000; $P = 0.051$ (Figure 2). Furthermore, three subgroup analyses were additionally carried out by ethnicity (Caucasian, Asian and Mexican Mestizo), age (children or adolescents, adults), and NAFLD (with or without).

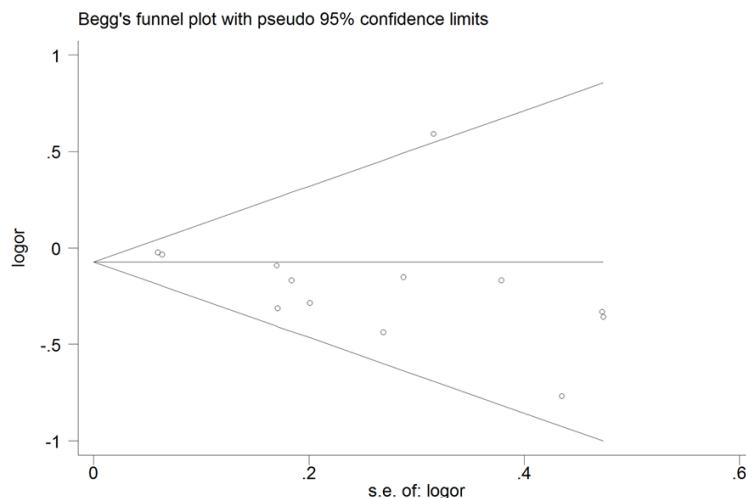


Figure 7. Funnel plots of publication bias of the overall pooled studies.

When combined data from Caucasians, the G allele, consisted of GG+CG, did not show an unfavorable effect compared to CC genotype (OR = 0.960, 95% CI: 0.885-1.042; $P = 0.327$). When combined data from Asian, the G allele was observed to have a favorable effect (OR = 0.707, 95% CI: 0.575-0.869; $P = 0.001$). Since only two studies referred to Mexican Mestizo, ethnicity stratification analyses were not established (**Figure 3**).

For adults group, six studies including 8 subgroups evaluated the association between rs738409 polymorphism and overweight/obesity risk. Since one study [2] was unavailable to separate children or adults from the population, it was not included in the comparison. However, no significant association between rs738409 and the risk of obesity was identified in adults (OR = 0.950, 95% CI: 0.877-1.030; $P = 0.215$). For children or adolescents group, similar results were observed from three studies including 4 subgroups (OR = 0.825, 95% CI: 0.669-1.017; $P = 0.072$) (**Figure 4**).

Among 10 incorporate studies of this meta-analysis, four of them including 4 subgroups were NAFLD patients. The risk of overweight/obesity was not related to PNPLA3 polymorphism both in patients with NAFLD or without NAFLD (OR = 0.665, 95% CI: 0.436-1.014; $P = 0.058$; OR = 0.940, 95% CI: 0.872-1.013; $P = 0.103$, respectively) (**Figure 5**).

Heterogeneity analysis

There was no significant heterogeneity for the pooled analysis ($n = 10$, subgroups = 13). In

the subgroup analyses, heterogeneity was not significant for the risk of overweight/obesity in Caucasians ($P = 0.893$, $I^2 = 0$) and Asians ($P = 0.924$, $I^2 = 0$). In addition, the between-study heterogeneity was not significant in adults ($P = 0.126$, $I^2 = 38.1\%$) and children or adolescents ($P = 0.829$, $I^2 = 0$). Furthermore, no significant heterogeneity was observed between studies in NAFLD patients ($P = 0.771$, $I^2 = 0$) and healthy patients ($P = 0.221$, $I^2 = 25.1\%$).

Sensitivity analysis

The influence of a single study on the overall meta-analysis was performed by sequential omission of every study respectively. No significant difference was showed when omitted any study; thus, our results were reliable (**Figure 6**).

Publication bias

Publication bias was examined by using Begg's test and Eggers' test respectively. The funnel plot did not reveal any evidence of an obvious asymmetry (**Figure 7**). The P values of Begg's test and Egger's test were 0.200 and 0.087, respectively. No significant publication bias was detected in our meta-analysis.

Discussion

The PNPLA3 gene (rs738409) has garnered much research attention recently. This SNP exhibits a C-to-G transition generating an amino acid substitution of isoleucine to methionine, was initially one of the potential candidate genes related to NAFLD susceptibility in patients with G allele [7]. Several replication studies have also replicated an association between the rs738409 polymorphism and NAFLD risk [28-30]. It is strongly associated with factors such as aging, obesities, and diabetes [31]. Consistent with this result, some following studies also demonstrated an association between the rs738409 polymorphism and obesity or overweight and less clearly with insulin resistance in the general population [7, 22, 32]. Also, in liver transplant recipients, PNPLA3 non-CC genotype was independently associ-

ated with obesity at 3 years post-transplant [12]. Therefore, this SNP seems not only to be a risk factor in the development of liver fat content but also overweight/obesity.

In the present study, we performed a meta-analysis included 10 studies involving 14,220 patients to evaluate the association of the PNPLA3 gene rs738409 polymorphism with overweight/obese patients. In this meta-analysis, we found no evidence that PNPLA3 rs738409 the G allele was associated with risk of overweight/obesity in either adults or children/adolescents, despite the fact that whether patients carried NAFLD or not. Ethnicity stratification analyses revealed that rs738409G allele was not associated with overweight/obesity in Caucasians. However, in our Asian-based samples of 798 cases and 1,644 controls, we observed that rs738409G allele had a favorable effect on lower risk of overweight/obesity. It was inconsistent with previous studies for rs738409 G allele (GG+GC) frequently to be adverse genotypes in metabolic disorders [32]. Results could be explained by several factors. On the one hand, the etiology of overweight/obesity is complex and the rs738409 in I148M may interact with other risk factors, such as differences in diet and physical activity (PA) behaviors, not assessed in these studies. On the other hand, the polymorphisms in PNPLA3 may have a complex interactive effect to increase both adiponutrin activity and overweight/obesity risk.

Some limitations of this study should be discussed. First, meta-analysis reflects the methodological problems of the included studies. Second, only published studies were included in the present meta-analysis, which may bring publication bias although Begg's test and Egger's test of publication bias turned out to be acceptable. Furthermore, overweight/obesity would be interpreted for multiple influence variables known or unknown. In this analysis, we could not adjust several potential factors for very limited data were reported and not available to compare.

In conclusion, the present meta-analysis, which included 10 independent studies containing 13 subgroups, has shown that the PNPLA3 gene rs738409 polymorphism was not associated with an enhanced risk of overweight/obesity among Caucasians, neither adults nor children

or adolescents. However, there is still a need for further research on this topic, including screening for etiological relationships between the other potential factors, and the susceptibility toward overweight/obesity.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jiping Li, Department of Nursing, West China Hospital, Sichuan University, 37 Guoxue Xiang, Chengdu 610041, Sichuan, China. Tel: +86-18980601058; E-mail: jiping_li_hx@163.com

References

- [1] Westley RL, May FE. A twenty-first century cancer epidemic caused by obesity: the involvement of insulin, diabetes, and insulin-like growth factors. *Int J Endocrinol* 2013; 2013: 632461-632461.
- [2] Mangge H, Almer G, Truschnig-Wilders M, Schmidt A, Gasser R, Fuchs D. Inflammation, adiponectin, obesity and cardiovascular risk. *Curr Med Chem* 2010; 17: 4511-4520.
- [3] Nguyen DM and El-Serag HB. The epidemiology of obesity. *Gastroenterol Clin North Am* 2010; 39: 1-7.
- [4] Visscher PM, Brown MA, McCarthy MI and Jian Y. Five years of GWAS discovery. *Am J Hum Genet* 2012; 90: 7-24.
- [5] Imen B, Asma O, Jihène R, Lamia R, Amani O, Nabila BR, Naoufel N, Ahmed BA and Ali B. Relationship between leptin G2548A and leptin receptor Q223R gene polymorphisms and obesity and metabolic syndrome risk in tunisian volunteers. *Genet Test Mol Biomarkers* 2012; 16: 726-733.
- [6] Pereira TV, Mingroni-Netto RC, Yamada Y. ADRB2 and LEPR gene polymorphisms: synergistic effects on the risk of obesity in Japanese. *Obesity (Silver Spring)* 2011; 19: 1523-1527.
- [7] Romeo S, Kozlitina J, Xing C, Pertsemidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nature Genet* 2008; 40: 1461-1465.
- [8] Wilson PA, Gardner SD, Lambie NM, Commans SA and Crowther DJ. Characterization of the human patatin-like phospholipase family. *J Lipid Res* 2006; 47: 1940-1949.
- [9] Yuexin Z, Wen C, Jiangmei S, Lei M, Bei Z, Qin X, Lijuan Z and Hua Y. Association between the PNPLA3 I148M polymorphism and non-alcoholic fatty liver disease in the Uygur and Han ethnic groups of Northwestern China. *PLoS One* 2014; 9: e108381.

- [10] Mangge H, Baumgartner BG, Zelzer S, Prüller F, Schnedl WJ, Reininghaus EZ, Haybaeck J, Lackner C, Stauber R, Aigner E, Weghuber D. Patatin-like phospholipase 3 (rs738409) gene polymorphism is associated with increased liver enzymes in obese adolescents and metabolic syndrome in all ages. *Aliment Pharmacol Ther* 2015; 42: 99-105.
- [11] Johansson LE, Lindblad U, Larsson CA, Råstam L, Ridderstråle M. Polymorphisms in the adiponutrin gene are associated with increased insulin secretion and obesity. *Eur J Endocrinol* 2009; 159: 577-583.
- [12] Watt KD, Dierkhising R, Fan C, Heimbach JK, Tillman H, Goldstein D, Thompson A, Krishnan A and Charlton MR. Investigation of PNPLA3 and IL28B genotypes on diabetes and obesity after liver transplantation: insight into mechanisms of disease. *Am J Transplant* 2013; 13: 2450-2457.
- [13] Ioannidis JPA, Paolo B, Julian L, O'Brien TR, Uitterlinden AG, Paolo V, Balding DJ, Anand C, Dolan SM and W Dana F. Assessment of cumulative evidence on genetic associations: interim guidelines. *Int J Epidemiol* 2008; 37: 120-132.
- [14] Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol* 2010; 25: 603-605.
- [15] Higgins JP, Thompson SG, Deeks JJ and Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; 327: 557-560.
- [16] Mantel N. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst* 1959; 22: 639-640.
- [17] Dersimonian R and Nan L. Meta-analysis in clinical trials. *Control Clin Trials* 1986; 7: 177-188.
- [18] Galbraith RF. A note on graphical presentation of estimated odds ratios from several clinical trials. *Stat Med* 1988; 7: 889-894.
- [19] Nishioji K, Mochizuki N, Kobayashi M, Kamaguchi M, Sumida Y, Nishimura T, Yamaguchi K, Kadotani H and Itoh Y. The impact of PNPLA3 rs738409 genetic polymorphism and weight gain ≥ 10 kg after age 20 on non-alcoholic fatty liver disease in non-obese Japanese individuals. *PLoS One* 2015; 10: e0140427.
- [20] Oniki K, Saruwatari J, Izuka T, Kajiwarra A, Morita K, Sakata M, Otake K, Ogata Y and Nakagawa K. Influence of the PNPLA3 rs738409 polymorphism on non-alcoholic fatty liver disease and renal function among normal weight subjects. *PLoS One* 2015; 10: e0132640.
- [21] Shang XR, Song JY, Liu FH, Ma J and Wang HJ. GWAS-identified common variants with non-alcoholic fatty liver disease in Chinese children. *J Pediatr Gastroenterol Nutr* 2015; 60: 669-74.
- [22] Nikolaj Thure K, Niels G, Karina B, Martin F, Kristine FR, Camilla Helene S, Torben JR, Pernille P, Daniel Rinse W and Allan V. The PNPLA3 rs738409 G-allele associates with reduced fasting serum triglyceride and serum cholesterol in danes with impaired glucose regulation. *PLoS One* 2012; 7: 109-111.
- [23] Larrieta-Carrasco E, León-Mimila P, Villarreal-Molina T, Villamil-Ramírez H, Romero-Hidalgo S, Jacobo-Albavera L, Gutiérrez-Vidal R, López-Contreras BE, Guillén-Pineda LE, Sánchez-Muñoz F, Bojalil R, Mejía-Domínguez AM, Méndez-Sánchez N, Domínguez-López A, Aguilar-Salinas CA, Canizales-Quinteros S. Association of the I148M/PNPLA3 variant with elevated alanine transaminase levels in normal-weight and overweight/obese Mexican children. *Gene* 2013; 520: 185-188.
- [24] Lallukka S, Sevastianova K, Perttilä J, Hakkarainen A, Orho-Melander M, Lundbom N, Oikkonen VM and Yki-Järvinen H. Adipose tissue is inflamed in NAFLD due to obesity but not in NAFLD due to genetic variation in PNPLA3. *Diabetologia* 2013; 56: 886-892.
- [25] Viitasalo A, Pihlajamaki J, Lindi V, Atalay M, Kaminska D, Joro R and Lakka TA. Associations of I148M variant in PNPLA3 gene with plasma ALT levels during 2-year follow-up in normal weight and overweight children: the PANIC Study. *Pediatr Obes* 2015; 10: 84-90.
- [26] Larrieta-Carrasco E, Acuña-Alonzo V, Velázquez-Cruz R, Barquera-Lozano R, León-Mimila P, Villamil-Ramírez H, Menjivar M, Romero-Hidalgo S, Méndez-Sánchez N, Cárdenas V, Bañuelos-Moreno M, Flores YN, Quiterio M, Salmerón J, Sánchez-Muñoz F, Villarreal-Molina T, Aguilar-Salinas CA, Canizales-Quinteros S. PNPLA3 I148M polymorphism is associated with elevated alanine transaminase levels in Mexican Indigenous and Mestizo populations. *Mol Biol Rep* 2014; 41: 4705-4711.
- [27] Stojkovic IA, Ericson U, Rukh G, Riddestråle M, Romeo S and Orho-Melander M. The PNPLA3 Ile148Met interacts with overweight and dietary intakes on fasting triglyceride levels. *Genes Nutr* 2014; 9: 200-209.
- [28] Sookoian S and Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology* 2011; 53: 1883-1894.
- [29] Santoro N, Kursawe R, D'Adamo E, Dykas DJ, Zhang CK, Bale AE, Calí AM, Narayan D, Shaw MM and Pierpont B. A common variant in the patatin-like phospholipase 3 gene (PNPLA3) is

- associated with fatty liver disease in obese children and adolescents. *Hepatology* 2010; 52: 1281-1290.
- [30] Luca V, Anna A, Enrico G, Andrea B, Benedetta DM, Arianna A, Paola D, Silvia F and Valerio N. I148M patatin-like phospholipase domain-containing 3 gene variant and severity of pediatric nonalcoholic fatty liver disease. *Hepatology* 2010; 52: 1274-1280.
- [31] Ji YL, Kang MK, Lee SG, Yu E, Lim YS, Han CL, Chung YH, Lee YS and Suh DJ. Prevalence and risk factors of non-alcoholic fatty liver disease in potential living liver donors in Korea: a review of 589 consecutive liver biopsies in a single center. *J Hepatol* 2007; 47: 239-244.
- [32] Konstantinos K, Andreas P, Fausto M, Jürgen M, Silvia W, Ingmar KN, Alfred KN, Fritz S, Andreas F and Hans-Ulrich HR. Dissociation between fatty liver and insulin resistance in humans carrying a variant of the patatin-like phospholipase 3 gene. *Diabetes* 2009; 58: 2616-2623.