Int J Clin Exp Med 2017;10(5):8429-8437
www.ijcem.com /ISSN:1940-5901/IJCEM0028244

Original Article

Apolipoprotein C3 polymorphisms C482T and T455C are not related to the susceptibility of non-alcoholic fatty liver disease: a meta-analysis

Hong Li, Hong Zhang, Hongfang Yang, Yingying Xu, Yuqian Jiao, Bingqian Wu, Weifeng Xiang, Shejun Gao

Department of Clinical Laboratory, The Fourth Hospital of Hebei Medical University, Shijiazhuang 050035, China

Received March 15, 2016; Accepted June 12, 2016; Epub May 15, 2017; Published May 30, 2017

Abstract: The aim of this study was to meta-analyze the association between apolipoprotein C3 gene (APOC3) polymorphism and the susceptibility of non-alcoholic fatty liver disease (NAFLD). Several databases, including PubMed, Embase, SpringerLink, Wanfang and Chinese National Knowledge Infrastructure (CNKI) were searched for relevant studies published before February 1, 2016. Hardy-Weinberg equilibrium (HWE) test was performed by using Stata11.0. The odds ratio (OR) and the 95% confidence interval (CI) were used as the effect sizes (ESs) to assess the association. Revman 5.2 software was used to pool the ESs. This meta-analysis included nine studies published during 2010-2014. There were 3977 subjects containing 2111 cases and 1866 controls. The results for both APOC3 C482T and T455C under all models (P>0.05) or the combined model (OR=1.00, 95% CI: 0.84-1.19, P=0.98) were not significant. These results indicate that there is no association between APOC3 polymorphisms (C482T and T455C) and NAFLD susceptibility.

Keywords: Apolipoprotein C3, polymorphism, non-alcoholic fatty liver disease, meta-analysis

Introduction

Nonalcoholic fatty liver disease (NAFLD), including nonalcoholic steatohepatitis (NASH) and fibrosis, is a chronic liver disease related to hepatic insulin resistance metabolic syndrome and diabetes [1]. Prevalence of NAFLD differs with race and ethnicity in the world (3%-24% [2]), more common in Asia-Pacific region [3, 4]. Polymorphisms of the NAFLD-related genes involving lipid handling, insulin signaling, and oxidative stress have attracted extensive attentions [5].

It's reported that apoprotein (APO) B/AI ratio is independently associated with NAFLD in non-diabetic subjects [6]. Recently, scholars have found that polymorphisms of APOC3 are associated with the susceptibility of NAFLD among non-Asian Indian men [1]. Afterward, more relative studies were reported on the association in different populations in the world [7-14]. Most studies that assessed the association between APOC3 and NAFLD focused on the polymorphic site of C482T (rs2854117) and T455C (rs2854116). Zhang *et al.* performed a meta-analysis [15], however, they emphasized on the combined effect of both C482T and T455C in NAFLD but not the separate effect. In addition, they also included the study of Verrijken *et al.* [10] who only assessed the C482T, which limited the application of this meta-analysis. What's more, subsequent studies have drewn different conclusions that the APOC3 (T455C) genetic variation is involved in NAFLD [7, 9] and the T allele carriers were more susceptible to metabolic disorder [9].

Thus, there is still inconsistent conclusion in the association between APOC3 and NAFLD especially the T455C, and it is necessary to update the meta-analysis. This study was therefore performed to further explore the association between APOC3 polymorphisms of C482T and T455C, or both and NAFLD susceptibility by introducing more new studies.

Materials and methods

Study search

The databases of PubMed, Embase, SpringerLink, Wanfang, and Chinese National Knowledge Infrastructure (CNKI) were searched accor-

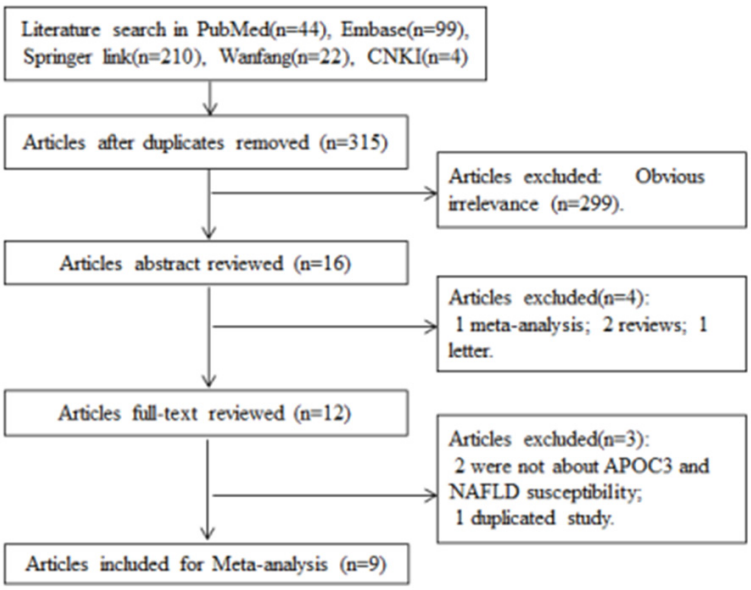


Figure 1. Literature search and study selection.

ding to the following strategy: ((apolipoprotein C3) OR APOC3 OR (APOC 3)) AND ((liver fat) OR (nonalcoholic fatty liver disease) OR (non-alcoholic fatty liver disease) OR NAFLD OR steatosis OR NASH OR (non-alcoholic steatohepatitis)) AND (polymorphism OR polymorphisms OR genetic OR variant OR variants). The search was up to February 1, 2016 with no language restriction. Besides, the authors also manually searched print documents. Relative reviews and references of the included studies were also read for more relative studies.

Study selection

The inclusion criteria for study selection were as follows: 1) the study was designed case-control study with NAFLD patients as casesand with non-NAFLD people as controls; 2) study assessed the correlation between the polymorphism of APOC3 gene (C482T and T455C) and theincidence of NAFLD; 3) study reported thegenotyping data or these data could be calculated according to the reported data.

The exclusion criteria were as follows: 1) the study included family members in both case and control groups was removed; 2) review, letter, comment and other non-original search were excluded; 3) if there were reduplicative publications or the data of one same crowd used in multiple studies, only the study with most complete information was included. Two authors (A and B) independently screened the studies according to the selection criteria.

Data abstraction and quality assessment

Two authors (B and C) independently abstracted the data according to a pre-designed table, including the name of the first author, the year of publication, the region that the research conducted, the age, sex and race of the subjects, sample sizes of the case and control group, source of controls, and the genotyping data. After completion of extraction, they exchanged the tables, and any inconsistent was resolved by discussion.

Study quality assessment of the included studies was performed by using the criteria of Clark *et al.* [16] which contained 10 items with 1 item scoring 1 point. The study with scores ≥8 points was considered high quality; 5-7 points, moderate; and <5, low quality [17].

Statistical analyses

Firstly, Hardy-Weinberg equilibrium (HWE) was examined with $P<0.05$ as significant disequilibrium by using Stata11.0 software (STATA, College Station, TX, USA). Secondly, odds ratios (ORs) and 95% confidence intervals (CI) were used as the effect sizes (ESs) to assess the association between APOC3 gene polymorphism and NAFLD susceptibility. Data statistics contained 3 parts: 1) both C482T and T455C and NAFLD susceptibility, WT (wild-type) *vs.* POL (polymorphic allele of C482T and T455C) [13]; 2) C-482T and NAFLD susceptibility under codominant model, dominant model, recessive model and additive model; 3) T455C and NAFLD susceptibility under codominant model, dominant model, recessive model and additive model.

Heterogeneity among studies was assessed by using Cochran Q statistic and I^2 test [18].

Table 1. Characteristics of included studies

Author, year	Country	Loci	Ethnicity	Type of control	Measurement of NAFLD	M/F (case, control)	Age	Genotyping	Case		Control	
									WT	POL	WT	POL
Niu TH, 2014	China	a, b	Asian	HB	US	185/205, 198/211	Adults	PCR	96	294	101	308
Hyysalo J, 2012	Finland	a, b	Caucasians	PB	H-MRS	220/197*	Adults	PCR	23	174	30	190
Peter A, 2012	Germany	a, b	Caucasians	HB	H-MRS	200/130*	Adults	PCR	40	65	65	150
Sentinelli F, 2011	Italy	a, b	Caucasians	HB	US	Not reported	Adults	RT PCR	49	66	25	30
Valenti L, 2011	Italian, UK	a, b	Caucasians	HB	Biopsy	301/457, 54/262	Adults, Pediatrics	PCR-RFLP	272	486	114	202

Author, year	Country	Loci	Ethnicity	Type of control	Measurement of NAFLD	M/F (case, control)	Age	Genotyping	Case			Control		
									CC	CT	TT	CC	CT	TT
Li MR, 2014a	China	a	Asian	HB	US	200/100, 200/100	Adults	PCR-RFLP	108	144	48	126	127	47
Niu TH, 2014	China	a	Asian	HB	US	185/205, 198/211	Adults	PCR	107	176	107	104	203	102
Puppala J, 2014	India	a	Asian	HB	US	92/58, 92/58	Adults	PCR-RFLP	55	57	38	62	46	42
Verrijken A, 2013	Belgium	a	Caucasians	HB	US and biopsy	Not reported	Adults	RT PCR	88	52	11	75	54	7

Author, year	Country	Loci	Ethnicity	Type of control	Measurement of NAFLD	M/F (case, control)	Age	Genotyping	Case			Control		
									TT	TC	CC	TT	TC	CC
Li MR, 2014b	China	b	Asian	HB	US	200/100, 200/100	Adults	PCR-RFLP	94	131	75	134	123	43
Niu TH, 2014	China	b	Asian	HB	US	185/205, 198/211	Adults	PCR	102	180	108	104	195	110
Puppala J, 2014	India	b	Asian	HB	US	92/58, 92/58	Adults	PCR-RFLP	44	75	31	60	81	9

a, C-482T; b, T-455C; HB, Hospital-based; PB, Population-based; US, Ultrasonographic; H-MRS, Hydrogen magnetic resonance spectroscopy; *, total participants; RT PCR, Real-Time polymerase chain reaction; PCR-RFLP, PCR-restriction fragment length polymorphism; WT, Wild-type homozygotes; POL, Polymorphic allele.

Table 2. Quality assessment of the included articles

Author	A	B	C	D	E	F	G	H	I	J	Sum
Li MR, 2014a	0	1	1	1	1	1	0	1	1	0	7
Li MR, 2014b	0	1	1	1	1	1	0	1	1	0	7
Niu TH, 2014	0	1	1	1	1	1	1	1	1	0	8
Puppala J, 2014	0	0	1	1	1	1	1	1	0	0	6
Verrijken A, 2013	0	1	1	1	1	1	1	1	1	0	8
Hyysalo J, 2012	1	0	1	1	1	1	0	1	1	0	7
Peter A, 2012	0	0	1	1	1	1	0	1	0	0	5
Sentinelli F, 2011	0	1	1	1	1	1	1	1	0	0	7
Valenti L, 2011	0	0	1	1	1	1	1	1	1	0	7

a: C-482T; b: T-455C. A: Control group; B: Hardy-Weinberg equilibrium; C: Case group; D: Primer; E: Reproducibility; F: Blinding; G: Power calculation; H: Statistics; I: Corrected statistics; J: Independent replication; Sum: Sum of quality assessment score; 1: Done; 0: Undone or unclear.

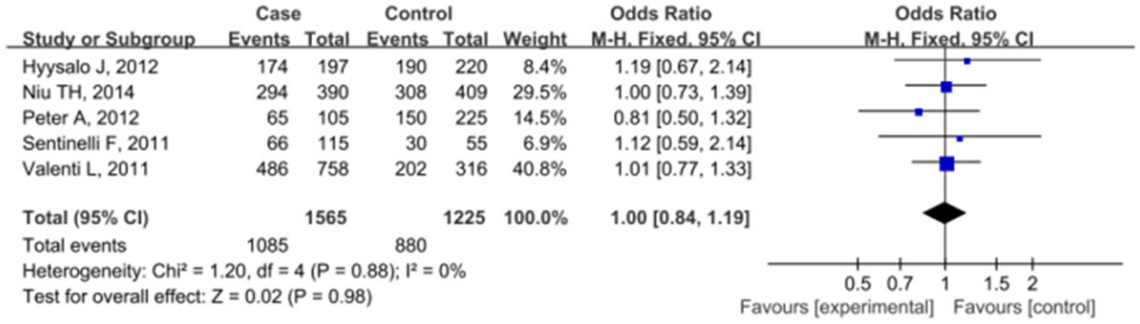


Figure 2. Forest plot of WT *vs.* POL for APOC3 and NAFLD susceptibility.

P<0.05 of Q statistic and/or I^2>50% of I^2 test indicated that there was significant heterogeneity and the random-effects analysis model was utilized to combined the ES. Otherwise (P≥0.05 and I^2≤50%), there was no heterogeneity and the fixed-effect analysis model was used. Combinations of the ESs were performed by using Review Manager5.2 software (Cochrane Collaboration, Oxford, UK). In addition, subgroup analysis based on the population was conducted. Publication bias was performed by using funnel plot method.

Results

Study search and selection

Figure 1 displayed the processes of study search and selection processes. According to the search strategy, the authors firstly obtained 44, 99, 210, 22 and 4 documents respectively on database PubMed, Embase, Springer link, Wanfang and CNKI. After removing the duplicates, there were 315 studies. By screening the title, 299 obviously unrelated studies were excluded. Among the remaining 16 studies, four studies were removed by reading the abstracts and three studies were excluded after reading the full text. No additional study was found by manual search. Finally, nine studies [7-14] were included in this study.

Characteristics of the included studies

There were totally 3977 subjects (2111 cases and 1866 controls) from the nine included studies involved in this meta-analysis. As shown in **Table 1**, the included studies were published during 2010-2014; five studies [8, 11-14] explored the combined effects of both C482T and T455C, and the sample with both site wild-type homozygotes was assigned into WT group and others into POL group (polymorphic allele). Four articles [7-10] reported the association between C482T and NAFLD and three [7-9] reported T455C and NAFLD. Excepting that the study by Valenti L, 2011 [13]

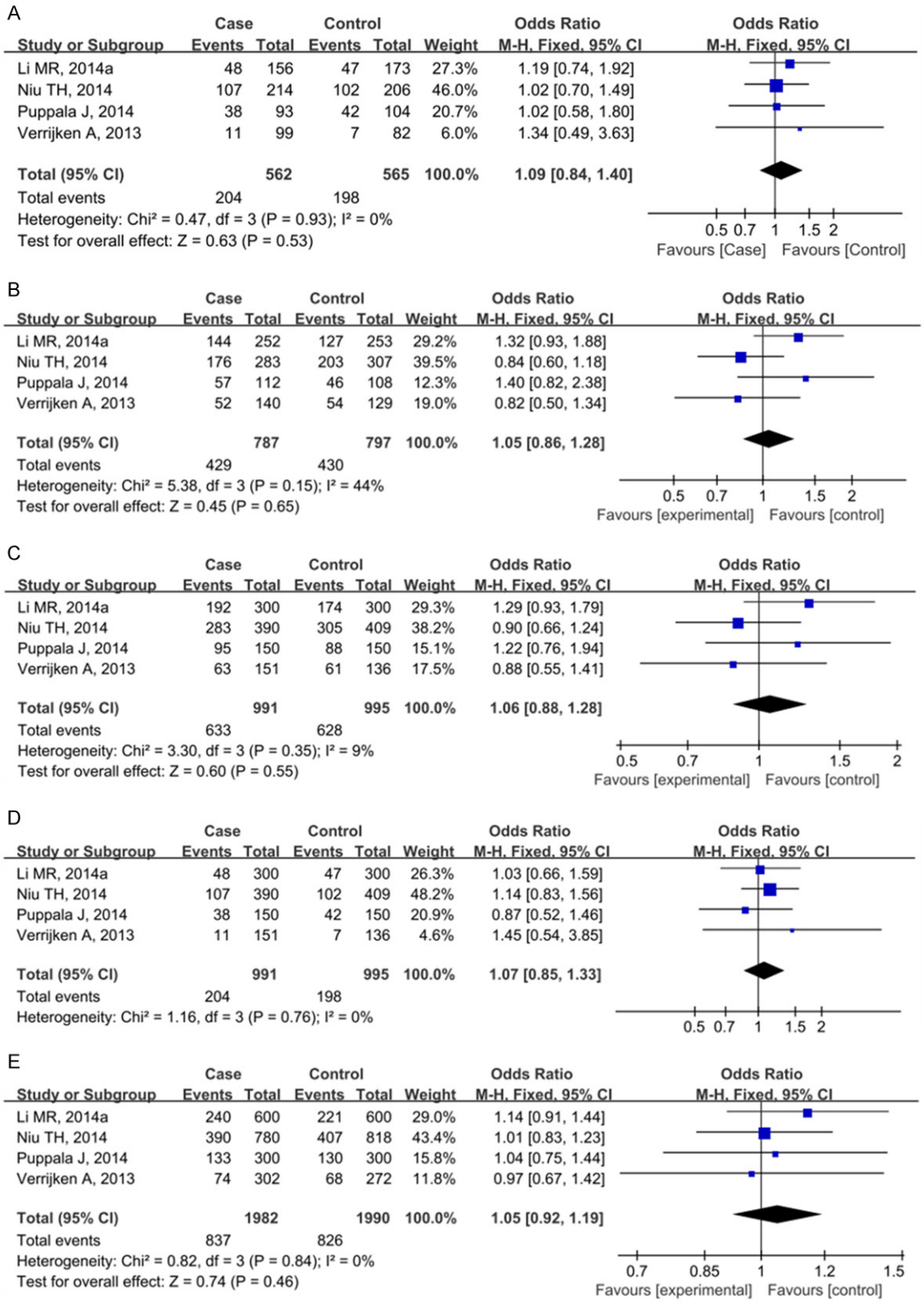


Figure 3. Forest plot of TT *vs.* CC (A), CT *vs.* CC (B), TT+CT *vs.* CC (C), TT *vs.* CT+CC (D), and T *vs.* C (E) for APOC3 C482T and NAFLD susceptibility.

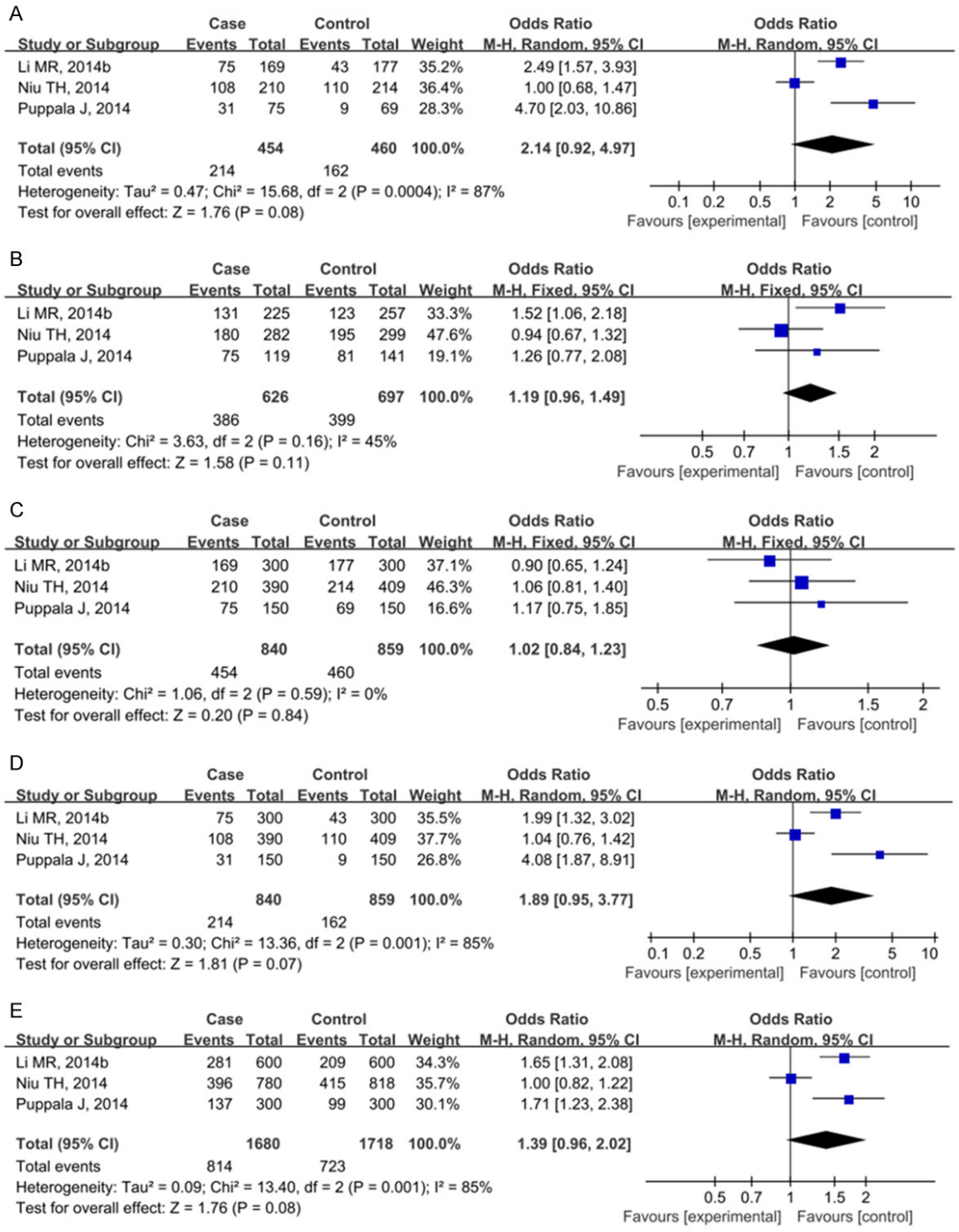


Figure 4. Forest plot of CC *vs.* TT (A), TC *vs.* TT (B), CC+TC *vs.* TT (C), CC *vs.* TC+TT (D), and C *vs.* T (E) for APOC3 T455C and NAFLD susceptibility.

contained some children, the subjects of all other studies were adults. The quality assessment indicated a relatively high quality of the included studies (5-8 points, **Table 2**).

WT vs. POL

As shown in **Figure 2**, there was no significant heterogeneity among studies and the (I^2=0%,

Table 3. Pooled results of subgroup analyses

Comparison	Asian		Caucasian	
	n	Effcet (95% CI)	n	Effcet (95% CI)
WT *vs.* POL	1	1.00 (0.73, 1.39)	4	1.00 (0.81, 1.23)
APOC3 C482T				
TT *vs.* CC	3	1.07 (0.82, 1.39)	1	1.34 (0.49, 3.63)
CT *vs.* CC	3	1.10 (0.88, 1.37)	1	0.82 (0.50, 1.34)
TT+CT *vs.* CC	3	1.10 (0.89, 1.34)	1	0.88 (0.55, 1.41)
TT *vs.* CT+CC	3	1.05 (0.83, 1.32)	1	1.45 (0.54, 3.85)
T *vs.* C	3	1.06 (0.92, 1.21)	1	0.97 (0.67, 1.42)

P=0.98), so the fixed-effect model was used. The result showed that there was no association between combined of C482T and T455C and NAFLD susceptibility (OR=1.00, 95% CI: 0.84-1.19, *P*=0.88).

C482T and NAFLD

The meta-analysis results of C482T and NAFLD were displayed in **Figure 3**. There were no significant heterogeneities among the studies for C482T and NAFLD under all models (TT *vs.* CC: I^2=0%, *P*=0.93; CT *vs.* CC: I^2=44%, *P*=0.15; TT+CT *vs.* CC; I^2=9%, *P*=0.35; TT *vs.* CT+CC: I^2=0%, *P*=0.76; T *vs.* C: I^2=0%, *P*=0.84) and the pooled results (by fixed effect model) were respectively OR=1.09, 95% CI: 0.84-1.40; OR=1.05, 95% CI: 0.86-1.28; OR=1.06, 95% CI: 0.88-1.28; OR=1.07, 95% CI: 0.85-1.33; OR=1.05, 95% CI: 0.92-1.19. These results indicated a lack of association between-APOC3 C482T and NAFLD susceptibility.

T455C and NAFLD

There was significant heterogeneity among the studies of CC *vs.* TT, CC *vs.* TC+TT, and C *vs.* T (*P*<0.05), but not among the studies of other models (*P*>0.05). The association between T455C polymorphism and NAFLD were not significant under all models (**Figure 4**, CC *vs.* TT, OR=2.14, 95% CI: 0.92-4.97; TC *vs.* TT, OR=1.19, 95% CI: 0.96-1.49; CC+TC *vs.* TT, OR=1.02, 95% CI: 0.84-1.23; CC *vs.* TC+TT, OR=1.89, 95% CI: 0.95-3.77; C *vs.* T, OR=1.39, 95% CI: 0.96-2.02).

Subgroup analysis and publication bias

Table 3 showed the results of the subgroup analysis. There was no significant association between APOC3 WT and POL and the NAFLD susceptibility among neither Asian nor Caucasian population (*P*>0.05). Similarly, there was no association between C482T polymorphism and NAFLD under each model (*P*>0.05).

The symmetrical funnel plot showed that there was no publication bias (Supplementary Figure 1).

Discussion

APOC3 is a component of very low density lipoprotein (VLDL) protein which is the precursor of LDL. APOC3 is thought to delay catabolism of triglyceride-rich particles by inhibiting lipoprotein lipase and hepatic lipase [19] which is related to the pathogenesis of NAFLD [20]. Thus, scholars believe that APOC3 plays a key role in NAFLD and they further explored whether the mutations of APOC3 affect the morbidity of NAFLD [21]. Recently, abundant studies are focused on the relationship of APOC3 polymorphisms and NAFLD susceptibility, however, there are conflicting opinions. Therefore, this meta-analysis was performed to systematically assess the association of APOC3 and NAFLD by pooling of the results of nine studies involving ethnicities of Asian, non-Asian Indian and Caucasians. The associations under multiple statistical models were all insignificant, which indicates that there was no association between APOC3 polymorphisms (C482T and T455C, or both) and NAFLD susceptibility in the general population.

Compared with the previous meta-analysis [15], the present study introduced three new studies [7, 9] for Asian population, although the results (OR=1.00, 95% CI: 0.84-1.19) were consistent with the previous meta-analysis that there was no significant association of APOC3 polymorphisms (combination of C482T and T455C) and NAFLD (OR=1.03, 95% CI: 0.89-1.22). In addition, we assessed the influence of C482T and T455C respectively, under different models and no correlation was found. What's more, subgroup analyses based on different population were conducted and no different results were found among Asian and Caucasian population. Besides, we conducted publication bias and the funnel plot promoted there was no publication bias. This further supports the conclusion that the polymorphisms of APOC3 do not induce a higher rate of NAFLD.

Most of the included studies suggest no association of C482T and NAFLD. However, there was a different opinion on T455C, which might contribute from the ethnicity variations and the small sample size. Although the overall results of the meta-analysis for T455C were not significant, there was also positive association in some included studies. For example, the study of Puppala *et al.* among [7] and Li *et al.* [22] among Asian population and showed a positive association. However, subgroup study by the Asian population did not find an association.

The advantages of this meta-analysis include 1) multiple statistical models were used to systematically assess the influences of the alleles, mutant gene type, and the combined effects of both C482T and T455C on the NAFLD susceptibility among different crowds; 2) the high quality of the included studies improved the reliability of the results of this study; and 3) the heterogeneity among studies was low. The major limitation of this study was the limited number of the enrolled studies, especially those independently discussed APOC3 C482T/T455C and NAFLD susceptibility and those for Caucasian, which may lead to insufficient statistical power. In addition, three different genotyping methods (PCR, RT-PCR and PCR-RFLP) were used in the included studies (**Table 1**), which may also affect the results of our meta-analysis. Nevertheless, due to the limited number of studies, stratification analysis based on this could not be employed.

In conclusion, there is no relationship between APOC3 polymorphisms (C482T and T455C) and NAFLD susceptibility. However, the findings need to verify by more large-scale studies due to the small number of included studies.

Disclosure of conflict of interest

None.

Address correspondence to: Shejun Gao, Department of Clinical Laboratory, Fourth Hospital of Hebei Medical University, 169 Tianshan Street, Shijiazhuang 050035, China. Tel: +86-0311-66696303; Fax: +86-0311-6077634; E-mail: gaodoc12@163.com

References

[1] Petersen KF, Dufour S, Hariri A, Nelson-Williams C, Foo JN, Zhang XM, Dziura J, Lifton RP and Shulman GI. Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. N Engl J Med 2010; 362: 1082-1089.

[2] Clark JM. The Epidemiology of Nonalcoholic Fatty Liver Disease in Adults. J Clin Gastroenterol 2006; 40 Suppl 1: S5-S10.

[3] Vernon G, Baranova A and Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther 2011; 34: 274-285.

[4] Fan JG and Farrell GC. Epidemiology of non-alcoholic fatty liver disease in China. J Hepatol 2009; 50: 204-210.

[5] Duseja A and Aggarwal R. APOC3 and PNPLA3 in non-alcoholic fatty liver disease: Need to clear the air. J Gastroenterol Hepatol 2012; 27: 848-851.

[6] Choe YG, Jin W, Cho YK, Chung WG, Kim HJ, Jeon WK and Kim BI. Apolipoprotein B/AI ratio is independently associated with non-alcoholic fatty liver disease in nondiabetic subjects. J Gastroenterol Hepatol 2013; 28: 678-683.

[7] Puppala J, Bhrugumalla S, Kumar A, Siddapuram SP, Viswa PD, Kondawar M, Akka J and Munshi A. Apolipoprotein C3 gene polymorphisms in Southern Indian patients with nonalcoholic fatty liver disease. Indian J Gastroenterol 2014; 33: 524-529.

[8] Niu TH, Jiang M, Xin YN, Jiang XJ, Lin ZH and Xuan SY. Lack of association between apolipoprotein C3 gene polymorphisms and risk of nonalcoholic fatty liver disease in a Chinese Han population. World J Gastroenterol 2014; 20: 3655-3662.

[9] Li MR, Zhang SH, Liao XH and Zhong BH. Relation between apolipoprotein C3 (-482 C>T) polymorphism and nonalcoholic fatty liver disease in the Han Chinese population. Practical Medicine (CHinese) 2014; 30: 2566-2569.

[10] Verrijken A, Beckers S, Francque S, Hilden H, Caron S, Zegers D, Ruppert M, Hubens G, Van Marck E, Michielsen P, Staels B, Taskinen MR, Van Hul W and Van Gaal L. A gene variant of PNPLA3, but not of APOC3, is associated with histological parameters of NAFLD in an obese population. Obesity (Silver Spring) 2013; 21: 2138-2145.

[11] Peter A, Kantartzis K, Machicao F, Machann J, Wagner S, Templin S, Konigsrainer I, Konigsrainer A, Schick F, Fritsche A, Haring HU and Stefan N. Visceral obesity modulates the impact of apolipoprotein C3 gene variants on liver fat content. Int J Obes (Lond) 2012; 36: 774-782.

[12] Hyysalo J, Stojkovic I, Kotronen A, Hakkarainen A, Sevastianova K, Makkonen J, Lundbom N, Rissanen A, Krauss RM, Melander O, Orho-

Melander M and Yki-Jarvinen H. Genetic variation in PNPLA3 but not APOC3 influences liver fat in non-alcoholic fatty liver disease. J Gastroenterol Hepatol 2012; 27: 951-956.

[13] Valenti L, Nobili V, Al-Serri A, Rametta R, Leathart JB, Zappa MA, Dongiovanni P, Fracanzani AL, Alterio A, Roviaro G, Daly AK, Fargion S and Day CP. The APOC3 T-455C and C-482T promoter region polymorphisms are not associated with the severity of liver damage independently of PNPLA3 I148M genotype in patients with nonalcoholic fatty liver. J Hepatol 2011; 55: 1409-1414.

[14] Sentinelli F, Romeo S, Maglio C, Incani M, Burza MA, Scano F, Coccia F, Cossu E, Leonetti F and Baroni MG. Lack of effect of apolipoprotein C3 polymorphisms on indices of liver steatosis, lipid profile and insulin resistance in obese Southern Europeans. Lipids Health Dis 2011; 10: 93.

[15] Zhang H, Chen L, Xin Y, Lou Y, Liu Y and Xuan S. Apolipoprotein C3 gene polymorphisms are not a risk factor for developing non-alcoholic Fatty liver disease: a meta-analysis. Hepat Mon 2014; 14: e23100.

[16] Clark MF, Baudouin SV. A systematic review of the quality of genetic association studies in human sepsis. Intensive Care Med 2006; 32: 1706-1712.

[17] Srivastava K, Srivastava A, Sharma KL, Mittal B. Candidate gene studies in gallbladder cancer: a systematic review and meta-analysis. Mutat Res 2011; 728: 67-79.

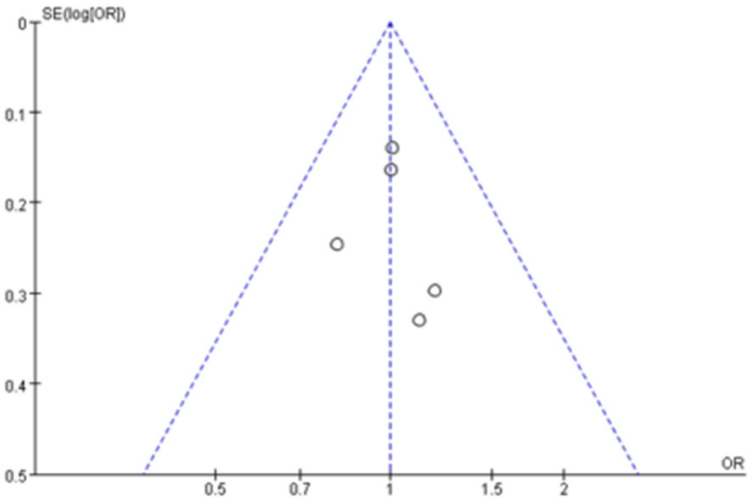
[18] Higgins JP, Thompson SG, Deeks JJ and Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003; 327: 557-560.

[19] Mendivil CO, Zheng C, Furtado J, Lel J and Sacks FM. Metabolism of very-low-density lipoprotein and low-density lipoprotein containing apolipoprotein C-III and not other small apolipoproteins. Arterioscler Thromb Vasc Biol 2010; 30: 239-245.

[20] Jiang ZG, Robson SC and Yao Z. Lipoprotein metabolism in nonalcoholic fatty liver disease. J Biomed Res 2013; 27: 1-13.

[21] Loomba R and Sanyal AJ. The global NAFLD epidemic. Nat Rev Gastroenterol Hepatol 2013; 10: 686-690.

[22] Li MR, Zhang SH, Chao K, Liao XH, Yao JY, Chen MH and Zhong BH. Apolipoprotein C3 (-455T>C) polymorphism confers susceptibility to nonalcoholic fatty liver disease in the Southern Han Chinese population. World J Gastroenterol 2014; 20: 14010-14017.



Supplementary Figure 1. Funnel plot of WT vs. POL for APOC3 and NAFLD susceptibility.