J Chin Med Assoc

ORIGINAL ARTICLE

A PRISMA-compliant meta-analysis of apolipoprotein C3 polymorphisms and nonalcoholic fatty liver disease

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Abstract

Background: The relationship between apolipoprotein C3 (*APOC3*) gene polymorphisms and nonalcoholic fatty liver disease (NAFLD) risk has been investigated in many studies, with inconclusive findings. This meta-analysis evaluated the effect of *APOC3* promoter region polymorphisms (–455T/C and –482C/T) on NAFLD susceptibility.

Methods: A comprehensive search of eligible studies up to October 2020 was performed on Medline, Embase, Web of Science, and Google Scholar databases. No restriction was imposed on language, publication date, or publication status. Odds ratios (ORs) with their 95% confidence intervals (CIs) were calculated to assess the combined effect sizes. The levels of heterogeneity, sensitivity, subgroup, and publication bias were analyzed subsequently.

Results: This meta-analysis included eight studies, consisting of 1,511 patients with NAFLD and 1,900 controls fulfilling the inclusion criteria and exclusion criteria. The pooled analysis showed significant associations between *APOC3* –455T/C polymorphism and NAFLD risk in allelic (OR = 1.33; 95% CI = 1.05-1.67), dominant (OR = 1.34; 95% CI = 1.04-1.72), and recessive (OR = 1.60; 95% CI = 1.06-2.40) models. Ethnicity-based stratification showed that –455T/C polymorphism was significantly associated with NAFLD risk in the non-Asian but not in the Asian population. No association was evident between –482C/T polymorphism and NAFLD risk.

Conclusion: Our findings suggest that *APOC3* promoter region polymorphism –455T/C may be associated with NAFLD risk in the non-Asian but not in the Asian population. Additional studies with other functional polymorphisms are needed to discover *APOC3* gene effects on NAFLD.

Keywords: Apolipoprotein C3; Meta-analysis; Nonalcoholic fatty liver disease; Polymorphism

1. INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is one of the most prevalent chronic liver diseases worldwide, affecting 25%–30% of the general population.[1,2] The fat accumulation in more than 5% of hepatocytes without excessive alcohol consumption (<30 g/day for men and <20 g/day for women) or secondary etiologies of hepatic steatosis is the main feature of NAFLD.[3] It contains a wide spectrum of hepatic disorders, ranging from simple steatosis to nonalcoholic steatohepatitis, which can further progress to advanced liver fibrosis and finally cirrhosis and hepatocellular carcinoma. NAFLD, a multifactorial liver disorder, is associated with insulin resistance, type 2 diabetes, obesity, metabolic syndrome, and cardiovascular disease.[4,5] Racial and regional differences in its prevalence and clinical features suggest that both genetic and environmental factors may influence individual susceptibility to NAFLD.[6]

Although the pathogenesis of NAFLD is still not clear, recent studies have focused on identifying NAFLD-related genes, particularly candidate genes involved in lipid metabolism, insulin regulation, and obesity. Numerous previous studies have evaluated the genetic influence of apolipoprotein C3 (*APOC3*) polymorphisms on the development of NAFLD.[7,8] *APOC3* encodes apolipoprotein C-III (Apo-CIII) protein. Apo-CIII is synthesized primarily in the liver and in minor quantities in the intestine and is found in triglyceride-rich and high-density lipoproteins.[9] APOC3 plays an important role in adipogenesis by inhibiting lipoprotein-lipase activity and attenuating the hepatic uptake of

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Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Journal of Chinese Medical Association. (2021) 84: 923-929.

Received May 11, 2021; accepted June 11, 2021.

doi: 10.1097/JCMA.0000000000000564.

triglyceride-rich particles.[10] Transgenic mice that overexpressed human *APOC3* were predisposed to NAFLD and insulin resistance, which provides a possible etiology for this common disorder in humans.[11]

APOC3 is located on chromosome region 11q23.[12] Single-nucleotide polymorphisms (SNPs) in *APOC3* were extensively studied in relation to liver disorders.[13] Of them, two polymorphisms (–455T/C and –482C/T) located in the insulin-response element of the promoter region are in linkage disequilibrium (LD) with the rs5128 polymorphism located in the 3′-untranslated region.[14] In addition, these two SNPs that are in strong LD with each other are associated with triglyceride levels in the plasma.[15] Therefore, these two promoter region SNPs are considered to be functional genetic variants that contribute to increased Apo-CIII levels. Apo-CIII delays the catabolism of triglyceride-rich particles and contributes to atherosclerosis and NAFLD.

A meta-analysis conducted by Zhang et al[16] reported no significant association between *APOC3* promoter polymorphisms and NAFLD risk; however, those findings regarding the relationship between *APOC3* polymorphisms (–455T/C or –482C/T) and NAFLD risk are still inconclusive.[17–24] Zhang et al did not investigate the relationship between NAFLD and each SNP polymorphism. Therefore, we conducted an updated systematic review and meta-analysis to investigate the influences of *APOC3* polymorphisms (–455T/C or –482C/T) on individual susceptibility to NAFLD.

2. METHODS

The protocol of our article was registered in International Prospective Register of Systematic Reviews (PROSPERO) websites on October 16, 2020 (ID: CRD42020209387).

2.1. Search strategy

A literature search without language restriction was conducted using the following electronic databases: Medline, Embase, Web of Science, and Google Scholar. The databases were searched from their inception to October 2020. The search strategy for eligible studies included the following keywords or MeSH terms in their title or abstract: ("APOC3" or "apolipoprotein C3"), ("NAFLD" or "nonalcoholic fatty liver disease" or "nonalcoholic steatohepatitis"), and ("SNP" or "single nucleotide polymorphism" or "polymorphism" or "mutation" or "genome-wide association study" or "genetic association study" or "genotype"). Furthermore, the authors manually searched the references of retrieved articles for relevant studies.

2.2. Inclusion and exclusion criteria

The inclusion criteria were described as followed: (1) a case-control or cohort study evaluating the association between *APOC3* polymorphisms and NAFLD risk; (2) *APOC3* –455T/C (rs2854116) or –482C/T (rs2854117) promoter region polymorphisms were evaluated; (3) studies included healthy individuals as controls; and (4) sufficient data in genotypic frequencies of cases and controls; (5) only the studies with participants above 18 years old were included. If a study met criterion (1) but the original genotype data were not provided, or the study met criterion (2) but was a narrative review, comment, or animal study, they were excluded from the present meta-analysis.

2.3. Data extraction and quality assessment

Two independent authors (C. H. Liu and B.-F. Chen) extracted the data separately. Disagreement resolution was achieved through consensus, and a senior author (Y. H. Wang) helped in the final decision making. The following information was prospectively extracted: first author's name, publication year, country, ethnicity, numbers of cases and controls, genotype data of cases and controls, and genotyping method. We used the Newcastle-Ottawa Scale (NOS) to assess the quality of eligible studies. NOS score ranged from 0 to 9, and the observational study quality was considered good if the score was >7.

2.4. Definition of outcomes

The primary endpoint of our study is the prevalence of each SNP (–455T/C or –482C/T) in *APOC3* among the NAFLD and control groups. NAFLD was mainly diagnosed through abnormal findings on abdominal sonography or histology with different criteria in included studies. The genotyping methods of SNP detection were diverse, including polymerase chain reaction (PCR) with restriction fragment length polymorphism (RFLP), PCR with sequencing, and real-time methods (TaqMan and MassARRAY). Because the true underlying inheritance mode of *APOC3* alleles in NAFLD outcomes is unknown, we examined SNP prevalence (–455T/C or –482C/T) with allelic, dominant, and recessive genetic models separately.

2.5. Statistical analysis

Using a goodness-of-fit χ^2 test, genotype frequency deviation of each SNP was assessed in controls based on Hardy-Weinberg equilibrium (HWE). The strength of the association between *APOC3* genetic polymorphisms and NAFLD risk was calculated using odds ratio (OR) with 95% confidence interval (CI). OR estimates of *APOC3* promoter region polymorphisms (–455T/C or –482C/T) were evaluated based on allelic, dominant, and recessive genetic models. Heterogeneity was evaluated through forest plot examination and statistically using χ^2-based Cochran's Q test and Higgins' I^2 heterogeneity index. Heterogeneity difference was regarded as statistically significant when Cochran's Q statistic had $p < 0.1$ or $I^2 > 50\%$. In studies with statistical heterogeneity, a random-effects model was used to calculate a pooled OR. Otherwise, a fixed-effects model was used. During concerns regarding heterogeneity, sensitivity analyses were performed by removing one study at a time to evaluate the effect of a single study. In addition, a subgroup analysis was conducted to assess the possible causes of heterogeneity according to ethnic difference: Asian or non-Asian population. Publication bias was graphically evaluated through a funnel plot analysis. All p values were two-tailed. All analyses were performed using RevMan (version 5.3), which was provided by Cochrane Collaboration.

3. RESULTS

Initially, 58 studies were identified through electronic database searches by using MEDLINE, EMBASE, Web of Science, and Google Scholar and manually searching. According to the inclusion and exclusion criteria, 17 potentially relevant studies were identified. After the exclusion of oral presentations and articles without original genotype data, eight studies with 1,511 cases and 1,900 controls were finally included in the present meta-analysis (Fig. 1).

3.1. Characteristics of eligible studies

The main characteristics of the eight included studies are shown in Table 1. Quality assessment of eligible studies with NOS score is demonstrated in Table 2. All included studies were published between 2013 and 2020. The populations in our study were diverse, including Caucasian, Egyptian, Han Chinese, and Indian populations. We classified the populations into two groups, Asian (Han Chinese, Indian) and non-Asian (Caucasian,

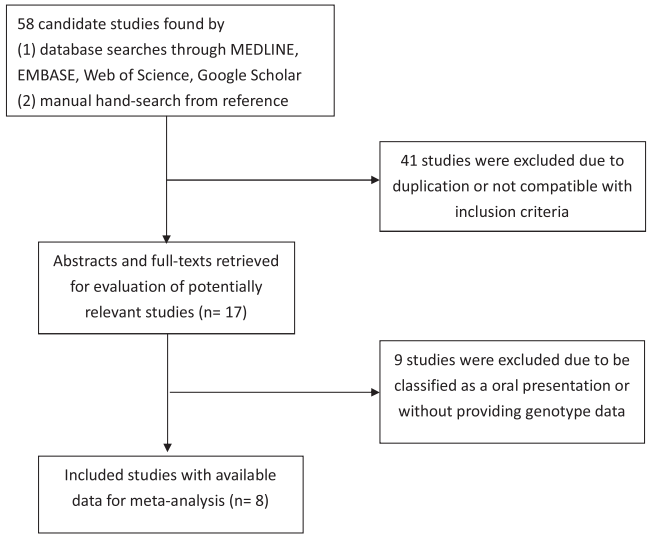

Fig. 1 Flow diagram of the study selection.

Egyptian, Indian). Due to the ethnic diversity of India, we classified the population of the study by Puppala et al[25] as mixed ethnicity (both Asian and non-Asian groups). Four studies used the conventional PCR with RFLP method for SNP genotyping, two studies used the conventional PCR with sequencing method, and the others used real-time methods (TaqMan and MassARRAY) for SNP genotyping.

The *APOC3* promoter region polymorphisms (–455T/C and –482C/T) are in strong LD with each other. Genotype distribution and allele frequencies of the included studies are shown in Table 3. Of them, Verrijken et al[21] assessed only one –482C/T polymorphism. Therefore, seven studies had an *APOC3* –455T/C polymorphism analysis, and four of those, plus the study by Verrijken et al[21] had a –482C/T polymorphism assessment. As shown in Table 3, five of the seven studies showed no deviation from HWE in the control population ($p > 0.05$). However, genotype distributions among controls in the studies of Puppala et al and Youssef et al violated HWE.[24,25]

3.2. Meta-analysis between *APOC3* promoter region polymorphisms and NAFLD

The main results between the *APOC3* gene polymorphism –455T/C and NAFLD risk are shown in Table 4. A random-effects model was applied to calculate a pooled OR of the –455T/C polymorphism, for its heterogeneity, was significant.

Table 2

Quality assessment of included studies with NOS

Author/ year	Selection				Comparability		Exposure			Total score
	a	B	C	d	e	F	g	h	i	
Verrijken 2013	+	–	–	+	+	+	+	+	–	6
Cai 2013	–	+	–	+	+	+	+	+	+	7
Li 2014	+	+	+	+	+	+	+	+	+	9
Niu 2014	–	+	+	+	+	+	+	+	+	8
Puppala 2014	+	+	+	+	+	–	+	+	+	8
Song 2017	–	+	+	+	+	+	+	+	+	8
Yang 2018	+	+	+	+	+	+	+	+	–	8
Youssef 2018	–	–	–	+	+	–	+	+	+	5

Quality assessment checklist:
a: Is the case definition adequate?
b: Representativeness of the cases
c: Selection of controls
d: Definition of controls
e: Study controls for ethnicity
f: Study controls for any additional factor
g: Ascertainment of exposure
h: Same method of ascertainment for cases and controls
i: Nonresponse rate
NOS = Newcastle-Ottawa scale.

The pooled analysis showed significant associations between *APOC3* –455T/C polymorphism and NAFLD risk in allelic (OR = 1.33; 95% CI = 1.05–1.67), dominant (OR = 1.34; 95% CI = 1.04–1.72), and recessive (OR = 1.60; 95% CI = 1.06–2.40) models (all $p = 0.02$) (Fig. 2).

The results between *APOC3* polymorphism –482C/T and NAFLD risk were not significant among all analyses. No association was evident between the polymorphism –482C/T and NAFLD risk in allelic, dominant, and recessive models under calculation with the fixed-effects model (Fig. 3).

To assess the potential influence of these study characteristics on the association between *APOC3* promoter polymorphisms and NAFLD risk, we performed subgroup analyses based on the study characteristics. When stratified based on ethnicity, the associations between the polymorphism –455T/C and NAFLD risk were significant in the non-Asian group (allelic OR: 1.92, 95% CI = 1.48–2.50, $p < 0.001$; dominant OR: 1.80, 95% CI = 1.24–2.63, $p = 0.002$; and recessive OR: 4.23, 95% CI = 2.39–7.50, $p < 0.001$) but not in the Asian group (allelic OR: 1.23, 95% CI = 0.99–1.53, $p = 0.06$; dominant OR: 1.27, 95% CI = 0.98–1.63, $p = 0.07$; and recessive OR: 1.40, 95% CI = 0.96–2.04, $p = 0.08$). No significant association was found between polymorphism –482C/T and NAFLD risk among either ethnical subgroup analyses.

Table 1

Basic characteristics of included studies in the present meta-analysis

First author	Year	Country	Ethnicity	Sample size (case/control)	Genotyping method
Verrijken	2013	Belgium	Caucasian	151/136	TaqMan SNP Genotyping Assays
Cai	2013	China	Han Chinese	193/209	PCR-RFLP
Li	2014	China	Han Chinese	300/300	PCR-RFLP
Niu	2014	China	Han Chinese	390/409	PCR and sequencing
Puppala	2014	India	Indian	150/150	PCR-RFLP
Song	2017	China	Han Chinese	130/251	MassARRAY SNP Genotyping
Yang	2018	China	Han Chinese	97/362	PCR and sequencing
Youssef	2018	Egypt	Egyptian	100/83	PCR-RFLP

RFLP = restriction fragment length polymorphism; SNP = single-nucleotide polymorphisms.

Table 3

Distribution of APOC3 gene promoter polymorphisms among included literatures

		–455 T>C					–482 C>T				
		Genotype			Allele (%)		Genotype			Allele (%)	
Author/year	Groups	T/T	T/C	C/C	C (%)	HWE (*p*)	C/C	C/T	T/T	T (%)	HWE (*p*)
Verrijken 2013	Case	–	–	–	–	–	88	52	11	24.5	–
	Control	–	–	–	–	–	75	54	7	25.0	0.49
Cai 2013	Case	54	99	40	46.4	–	73	105	25	38.2	–
	Control	80	95	34	39.0	0.52	71	86	31	39.4	0.57
Li 2014	Case	94	131	75	46.8	–	–	–	–	–	–
	Control	134	123	43	34.8	0.09	–	–	–	–	–
Niu 2014	Case	102	180	108	50.8	–	107	176	107	50.0	–
	Control	104	195	110	50.7	0.35	104	203	102	49.8	0.88
Puppala 2014	Case	44	75	31	45.7	–	55	57	38	44.3	–
	Control	60	81	9	33.0	0.01	62	46	42	43.3	<0.001
Song 2017	Case	44	63	23	41.9	–	–	–	–	–	–
	Control	88	117	46	41.6	0.52	–	–	–	–	–
Yang 2018	Case	39	46	12	36.1	–	–	–	–	–	–
	Control	135	177	50	38.3	0.51	–	–	–	–	–
Youssef 2018	Case	30	38	32	51.0	–	40	35	25	42.5	–
	Control	40	35	8	30.7	0.93	34	30	19	41.0	0.02

HWE = Hardy-Weinberg equilibrium.

Table 4

Meta-analysis of the association between APOC3 –455T/C and –482C/T polymorphisms and nonalcoholic fatty liver disease

				Allelic model		Dominant model		Recessive model	
Polymorphism	Group/ subgroup	Model	No. of studies	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
–455T/C (rs2854116)				C vs. T		TC+CC vs. TT		CC vs. TC+TT	
	Overall	Random	7	1.33 (1.05-1.67)	0.02	1.34 (1.04-1.72)	0.02	1.60 (1.06-2.40)	0.02
	Ethnicity								
	Asian	Random	6	1.23 (0.99-1.53)	0.06	1.27 (0.98-1.63)	0.07	1.40 (0.96-2.04)	0.08
	Non-Asian	Random	2	1.92 (1.48-2.50)	<0.01	1.80 (1.24-2.63)	<0.01	3.38 (2.04-5.60)	<0.01
–482C/T (rs2854117)				T vs. C		CT+TT vs. CC		TT vs. CT+CC	
	Overall	Fixed	5	1.00 (0.88-1.14)	0.96	0.99 (0.82-1.20)	0.96	1.02 (0.82-1.27)	0.87
	Ethnicity								
	Asian	Fixed	3	1.00 (0.87-1.16)	0.99	1.02 (0.82-1.27)	0.89	0.98 (0.77-1.25)	0.89
	Non-Asian	Fixed	3	1.03 (0.83-1.27)	0.81	1.04 (0.78-1.38	0.80	1.02 (0.70-1.48)	0.93

CI = confidence intervals; OR = odds ratio.

3.3. Sensitivity analysis and publication bias

To appraise the stability of results, sensitivity analyses were performed using the leave-one-out approach and recalculating the summary OR. The statistical significance of the association between the –455T/C polymorphism and NAFLD risk was lost in allelic, dominant, and recessive models after exclusion of the study by Li et al,[17] Puppala et al,[25] or Youssef et al.[24] The shapes of the funnel plots were symmetric, indicating the publication bias was low in the current meta-analysis ($p > 0.05$, Egger's test).

4. DISCUSSION

In the present study, we conducted a meta-analysis to evaluate the association between *APOC3* polymorphisms (–455T/C or –482C/T) and NAFLD risk. The pooled results indicated that the *APOC3* –455T/C polymorphism confers an increased NAFLD risk based on allelic, dominant, and recessive inheritance models. A subgroup analysis showed statistically significant associations between *APOC3* –455T/C polymorphism and NAFLD risk only in the non-Asian population. The association trends were altered if the study by Li et al,[17] Puppala et al,[25] or Youssef et al[24] was excluded from the sensitivity analysis.

The lipase-inhibiting *APOC3* SNPs (–455T/C and –482C/T) were considered related to an increased risk of hypertriglyceridemia, obesity, metabolic syndrome, and coronary heart disease.[26,27] Recently, these genetic variants were shown to be associated with susceptibility to NAFLD.[6] An in vitro promoter assay study demonstrated that these polymorphic sites at –455 and –482, which appear within a previously identified insulin-response element, prevent insulin binding and thus increase *APOC3* mRNA and protein levels.[15] Insulin resistance is an essential pathophysiological factor of NAFLD, which results in hepatic de novo lipogenesis and a reduction in adipose tissue lipolysis, with an increase in fatty acids in liver.[28] Therefore, variant alleles were proposed to lead to increased production of *APOC3* and inhibition of lipoprotein-lipase activity and triglyceride clearance, resulting in hypertriglyceridemia due to an increased hepatic uptake of circulating chylomicron-remnant particles, causing NAFLD.[6,29]

Several studies have evaluated the combined effect of *APOC3* polymorphisms on NAFLD. For instance, a meta-analysis conducted by Zhang et al in 2014[16] reported no association between *APOC3* promoter polymorphisms and NAFLD risk in different populations. Zhang et al[16] investigated a combined effect

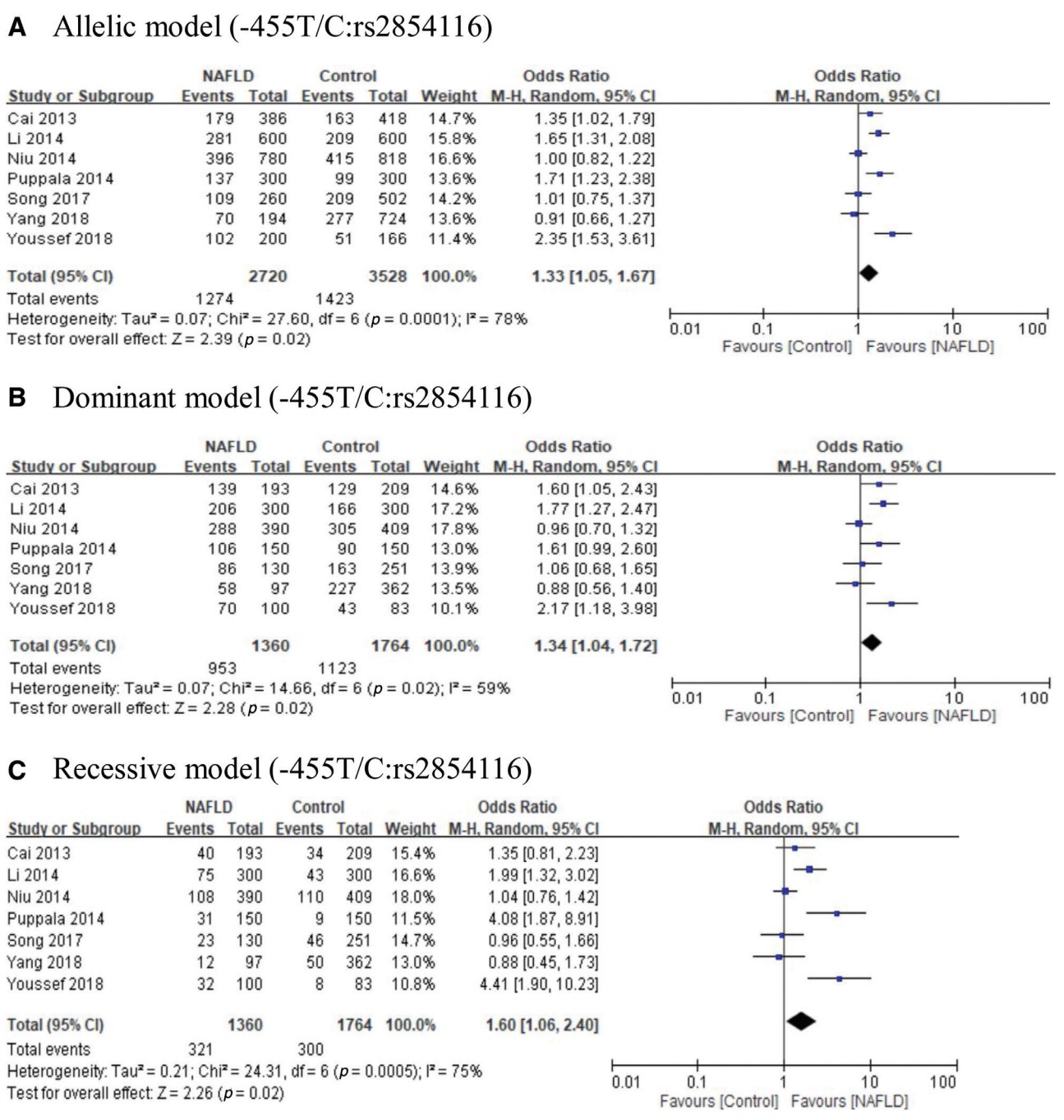

Fig. 2 Forest plots for the association between APOC3 –455T/C and nonalcoholic fatty liver disease under the (A) allelic, (B) dominant, and (C) recessive genetic model.

of both *APOC3* polymorphisms through a comparison of wild-type homozygotes (–455C/C and –482T/T) with carriers of one or more at-risk alleles (–455T and –482C). However, the function of these two SNPs may be independent.[6] Furthermore, the effect of an individual SNP was not thoroughly demonstrated. In our updated meta-analysis, one of the gene polymorphisms (–455T/C) was weakly correlated with NAFLD ($p = 0.02$). Furthermore, the –482C/T polymorphism showed no detectable effect on NAFLD.

The –455-polymorphism site is within a previously identified promoter insulin-response element. Lee et al[15] demonstrated that the variant sequence at the –455 site reduces the affinity for transcription factors that mediate insulin response and provides a potential explanation for the inability of the variant promoter allele to respond to insulin. Insulin resistance results in an increased delivery of free fatty acids to the liver. Moreover, insulin resistance is often accompanied by chronic low-grade inflammation and oxidation. Therefore, ectopic lipid accumulation and activated inflammation cascades increase susceptibility to hepatic injury and finally result in NAFLD.[28]

NAFLD is a major public health hazard globally. NAFLD prevalence varied greatly based on ethnicity, with the highest prevalence in Hispanics (45%–58%), followed by Caucasians (33%–44%), but the lowest prevalence in African Americans

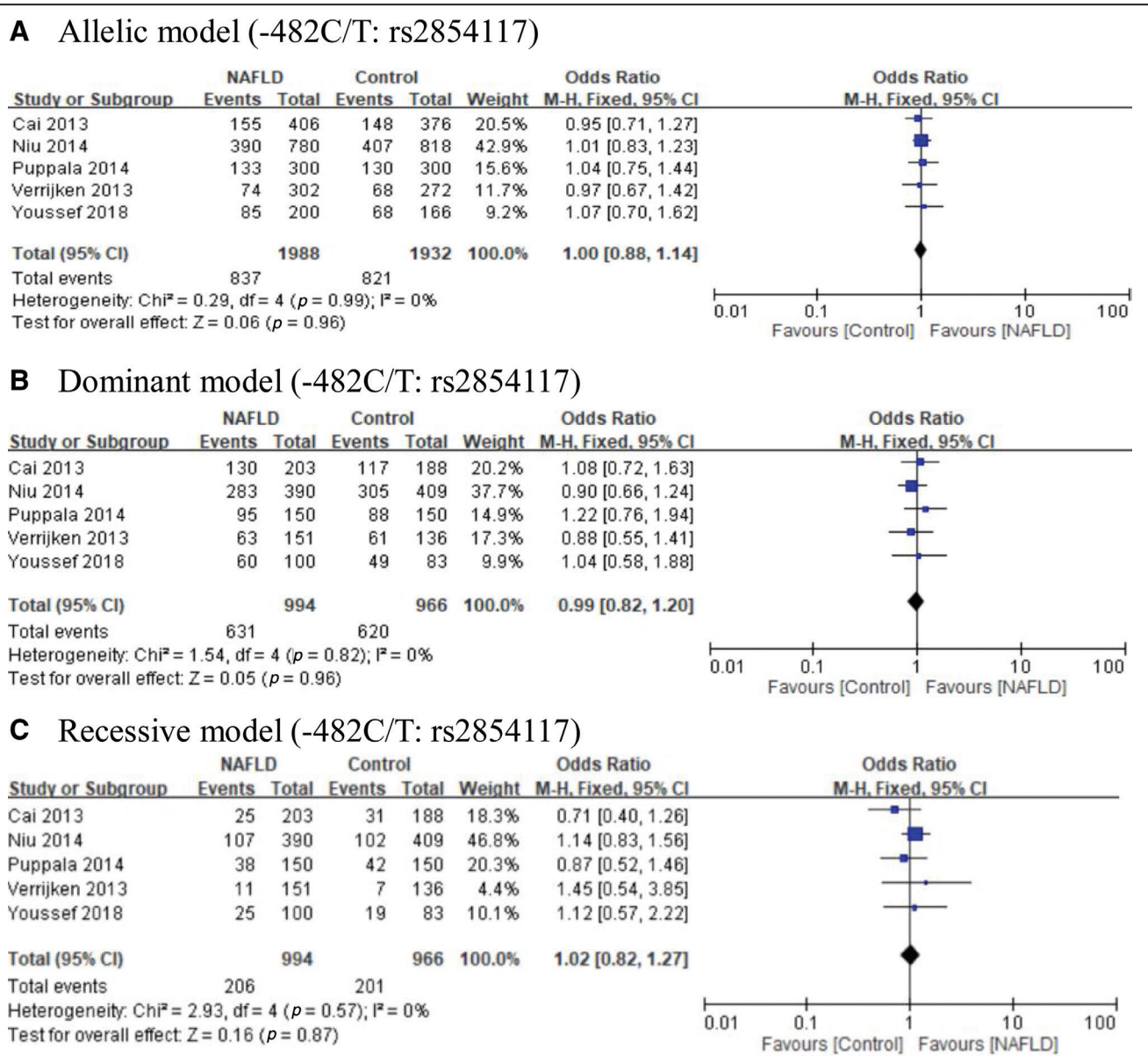

Fig. 3 Forest plots for the association between APOC3 –482C/T and nonalcoholic fatty liver disease under the (A) allelic, (B) dominant, and (C) recessive genetic model.

(24%–35%).[3,30,31] Therefore, we performed subgroup analyses based on the ethnicity of study participants and found that the –455C allele had a significant effect on NAFLD risk in non-Asian participants (Caucasian, Egyptian, and partial Indian) but not in Asian participants (Han Chinese and partial Indian). Among Asian subgroup, only Han Chinese population in the study by Li et al[17] showed a significant association in all the three genetic models, probably due to clear NAFLD diagnosis under abdominal sonography. The Indian population in the study by Puppala et al[25] and Egyptian and Caucasian populations in the study by Youssef et al[24] showed a significant association. Different genetic backgrounds and environments may contribute to ethnic disparities in NAFLD. Nevertheless, observational studies with clear diagnosis criteria of NAFLD are required to prove the ethnic difference.

It is worth to mention that adolescents and children were not involved in the present study because there were fewer pediatric studies investigated the association between NAFLD and polymorphisms of candidate genes. However, the issue may be important due to the growing numbers of obesity and NAFLD happened at the early age. Populations of adolescents and children are also more susceptible to genetic factors than adults. In a recent study conducted by Jain et al,[7] although the sample size was not large enough, they not only paid attention on Indian adolescents but also found the association between *APOC3* –455T/C polymorphism and the development of NAFLD.

Several limitations should be noted when interpreting our study results. First, this meta-analysis included a limited number of studies. Meanwhile, most of the participants were Asian. Therefore, the generalizability of our findings is uncertain, especially for non-Asian populations. Second, method heterogeneity was observed in NAFLD diagnosis; two studies[21,23] used liver histology, and the others used ultrasound.

Moreover, NAFLD diagnosis under ultrasound among included studies showed discrepancy. Furthermore, other factors such as age, sex, and ethnicity may introduce significant between-study heterogeneity. Third, we could not adjust for potential confounding factors, such as center settings, manipulating and interpreting skills of sonography, and species or doses of regimens while genotyping, which might have affected the accuracy in evaluating the effects of *APOC3* polymorphisms on the susceptibility to NAFLD.

In conclusion, our findings suggest that APOC3 –455T/C promoter region polymorphism may influence individual susceptibility to NAFLD, especially for the non-Asian population. Additional studies with other functional polymorphisms are needed to evaluate gene-gene interactions and the APOC3–NAFLD association.

ACKNOWLEDGMENTS

This work was supported by Shuang Ho Hospital [grant number106TMU-SHH-25] and Taipei Medical University, Taiwan [grant number TMU103-AE1-B08].

Supplemental Digital Content; http://links.lww.com/JCMA/A80

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://doi.org/10.1097/JCMA.0000000000000264.

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