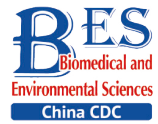


Original Article



Genetic Variations and Nonalcoholic Fatty Liver Disease: Field Synopsis, Systematic Meta-Analysis, and Epidemiological Evidence*

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Abstract

Objective To systematically summarize the published literature on the genetic variants associated with nonalcoholic fatty liver disease (NAFLD).

Methods Literature from Web of Science, PubMed, and Embase between January 1980 and September 2022 was systematically searched. Meta-analyses of the genetic variants were conducted using at least five data sources. The epidemiologic credibility of the significant associations was graded using the Venice criteria.

Results Based on literature screening, 399 eligible studies were included, comprising 381 candidate gene association, 16 genome-wide association, and 2 whole-exome sequencing studies. We identified 465 genetic variants in 173 genes in candidate gene association studies, and 25 genetic variants in 17 genes were included in the meta-analysis. The meta-analysis identified 11 variants in 10 genes that were significantly associated with NAFLD, with cumulative epidemiological evidence of an association graded as strong for two variants in two genes (*HFE*, *TNF*), moderate for four variants in three genes (*TM6SF2*, *GCKR*, and *ADIPOQ*), and weak for five variants in five genes (*MBOAT7*, *PEMT*, *PNPLA3*, *LEPR*, and *MTHFR*).

Conclusions This study identified six variants in five genes that had moderate to strong evidence of an association with NAFLD, which may help understand the genetic architecture of NAFLD risk.

Key words: Nonalcoholic fatty liver disease; Genetic association study; Genetic variant; Systematic review; Meta-analysis

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a rapidly growing health problem affecting approximately one-quarter of the world's population^[1]. It encompasses a range of clinical phenotypes, from hepatic steatosis to liver cirrhosis and even hepatocellular carcinoma^[2]. NAFLD has been linked to not only liver-related health issues and death but also a range of other health problems, including cardiovascular and chronic kidney diseases^[3].

NAFLD is a complex multifactorial disease involving genetic, metabolic, and environmental factors^[4-6]. Over the past three decades, more than 400 candidate gene association studies have been published on the associations between common variants and NAFLD risk, and some common variants, including *PNPLA3* rs738409, *TM6SF2* rs58542926, *GCKR* rs780094, have been studied frequently^[7-10]. An increasing number of genome-wide association studies (GWASs) and whole-exome sequencing (WES) have been conducted in recent years. GWASs provide a broader and unbiased approach for the discovery of genes involved in complex genetic traits, whereas WES studies can directly capture relevant variations not interrogated by common genotyping platform designs, including rare variants^[11]. Despite hundreds of genetic association studies published to date, results regarding the specific variant remain inconsistent, hindering our understanding of the genetic architecture of NAFLD. This may be attributed to small sample sizes in most studies, ethnic differences, and other factors. Meta-analysis is a powerful tool for increasing statistical strength and precision in detecting gene-disease associations^[12]. However, previous meta-analyses on NAFLD susceptibility have mainly focused on one genetic variant or genetic variants within one gene, and few meta-analyses have encompassed all relevant types of studies, including candidate gene association studies, GWASs, and WES studies. In addition, previous meta-analyses have not evaluated the epidemiological credibility of these associations^[13-15].

This study aimed to comprehensively summarize all genetic association studies of NAFLD, including candidate gene association, genome-wide association, and whole exome sequencing studies. This study will also conduct meta-analyses for genetic variants with sufficient available data, categorize related genes according to their genetic functions, and evaluate the epidemiological

credibility of these associations using the Venice criteria proposed by the Human Genome Epidemiology Network (HuGENet)^[16]. This study represents a comprehensive synthesis of genetic variants and functions associated with NAFLD, and will facilitate the interpretation of findings in the quest for genuine genetic susceptibility factors.

METHODS

Literature Searches and Study Eligibility

A comprehensive literature search was conducted in the Web of Science, PubMed, and Embase electronic databases to identify studies that analyzed genetic variants associated with NAFLD. The systematic search was first conducted on January 5, 2021, and updated on September 30, 2022. The detailed search terms are presented in Appendix A.

Studies included in this systematic review must satisfy the following criteria: (i) the study design must be a case-control, cohort, or cross-sectional association study in human subjects; (ii) the study must focus on the associations between genetic variants and NAFLD risk; (iii) NAFLD cases must have been diagnosed by clinicians, and meet the diagnostic criteria of NAFLD defined by the presence of steatosis in > 5% hepatocytes without secondary causes such as significant alcohol consumption, chronic viral hepatitis, long-term use of steatogenic medications, or monogenic hereditary disorders (including lecithin-cholesterol acyltransferase deficiency, cholesterol ester storage disease, Wolman's disease, etc); (iv) absence of hepatic steatosis in the control group; (v) if data or data subsets were published in more than one publication, the article with largest sample size was included; and (vi) article published in English. All records obtained through the database search were collected using EndNote software. After removing duplicates, the titles and abstracts were screened to exclude irrelevant studies, and the full texts of the remaining records were reviewed for eligibility. The reference lists of publications were also reviewed. Two reviewers (LI Ya Mei and XIAO Xiang) independently selected the studies.

Data Extraction and Study Quality Assessment

The following information was extracted independently by two reviewers from eligible studies: first author, year of publication, country of origin, ethnicity of the study population, diagnostic

criteria for NAFLD, sample size, source of controls, genotype methods, significant association (Yes/No), confounding factors adjusted in multivariate analysis models, and allele and genotype frequencies in cases and controls. Specifically, ethnicity was classified as Asian, Caucasian, Hispanic, African, or mixed, based on the ethnicity of at least 80% of the study population. The most recent gene names and variant accession numbers were used according to the National Center for Biotechnology Information (NCBI) database^[17].

Two reviewers independently assessed the quality of the included studies using the Newcastle–Ottawa Scale (NOS)^[18]. The NOS criteria evaluated the methodological quality of studies using a “star” rating system, with scores ranging from 0 star (worst) to 9 stars (best). Disagreements in data extraction and quality assessment were discussed among all the investigators and resolved by consensus.

Statistical Analysis

The locations, coding proteins, and functional annotation information of the genes identified in previous studies were obtained by searching the human gene database (<https://www.genecards.org/>). Genes were divided into eight categories based on their main related functions, including lipid synthesis and metabolism, insulin resistance and glucose metabolism, adipokines/adipokine receptors, energy metabolism and obesity, oxidative stress and antioxidants, inflammatory and immune responses, and liver fibrosis.

A meta-analysis was performed for genetic variants with data available from at least five independent studies. The GWAS-identified variants were not included in this meta-analysis because they have been convincingly replicated in many studies. Genotype distributions in the control subjects were tested for Hardy–Weinberg equilibrium using the chi-square (χ^2) test. Studies that did not conform to Hardy–Weinberg equilibrium were excluded from the meta-analysis. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using five genetic models: allele contrast model (mutant [M] allele versus wild [W] allele), heterozygous model (WM versus WW), homozygous model (MM versus WW), recessive model (MM versus WW + WM), and dominant model (WM + MM versus WW)^[19]. Random-effects models (Der Simonian–Laird method) were used in all meta-analyses^[20]. Cochrane’s *Q* test and I^2 index were used to assess heterogeneity across eligible studies^[21,22]. Meta-

analyses, stratified by ethnicity, were conducted for variants with at least two data sources in each subgroup. Funnel plots and Egger’s linear regression tests were used to assess potential publication bias in the included studies^[23]. All tests were two-sided, and *P* value < 0.05 was considered statistically significant. Sensitivity analyses were conducted to evaluate the influence of each study on the overall estimates. Meta-analyses were performed using the STATA version 12.0 software (Stata Corporation, College Station, TX, USA).

Assessment of Cumulative Evidence

The Venice criteria were applied to evaluate the epidemiological credibility of the significant associations identified in the meta-analysis^[16]. Credibility was defined as strong (A), moderate (B), or weak (C) in three categories: the amount of evidence, replication of the association, and protection from bias. The amount of evidence was graded by the sum of the test alleles or genotypes among the cases and controls in the meta-analysis: A grade of A for > 1,000, B for 100–1,000, and C for < 100. Replication of the association was graded using the heterogeneity statistic: A for $I^2 < 25\%$, B for I^2 between 25 and 50%, and C for $I^2 > 50\%$ ^[22]. Protection from bias was graded as A, if there was no observable bias and bias was unlikely to explain the presence of the association, B, if bias was present, and C, if bias was evident or likely to explain the presence of the association. We also considered the magnitude of association in the assessment of protection from bias, and a score of C was assigned to an association when the summary OR deviated less than 1.15-fold from the null unless the association had been replicated prospectively by several studies with no evidence of publication bias. Next, the cumulative epidemiological evidence for significant associations was considered strong if all three grades were A, moderate if all three grades were A or B, and weak if any grade was C.

RESULTS

Literature Searches and Study Characteristics

We identified 17,349 articles based on the literature searches, 11,391 non-duplicated articles were included in the title and abstract screening. Articles were excluded for non-human subjects ($n = 3,225$), irrelevant themes ($n = 2,904$), gene expression studies ($n = 1,759$), review articles ($n = 1,325$), duplicates ($n = 846$), or conference abstracts

($n = 548$), and the remaining 784 articles were included in the full-text review (Figure 1). In total, 399 eligible studies were identified, including 16 GWASs, 2 WES studies, and 381 candidate gene association studies. Of these, 205 studies with sufficient data were included in the meta-analysis of 25 variants of 17 genes.

Among the 16 GWASs, eight were conducted in the United States, four in Japan, two in South Korea, one in Australia, and one in Italy. Thirteen GWASs were conducted in adults, 2 studies were conducted in children, and 1 each in adults and children. The mean sample size was $5,710 \pm 7,595$ (ranged 234–27,374). At the same time, subsequent validation studies were performed followed by five GWAS studies. The basic characteristics of the GWASs are shown in Supplemental Table S1 (available in www.besjournal.com).

The characteristics of the 381 candidate gene association studies are presented in Supplemental Table S2 (available in www.besjournal.com). These studies analyzed 465 genetic variants in 173 candidate genes. The first candidate gene

association study for NAFLD was published in 1998 and the number of such studies has gradually increased in recent decades. Most studies were conducted in China ($n = 194$), followed by Italy ($n = 37$), Japan ($n = 21$), Turkey ($n = 18$), Iran ($n = 17$), India ($n = 16$), the United States ($n = 13$), Egypt ($n = 8$), and other countries ($n = 57$). The ethnic distribution was dominated by Asian ($n = 251$) and Caucasian ($n = 102$) populations. The vast majority of the studies included adults ($n = 349$), and the remaining 32 studies were conducted in children or a mixed population. The mean NOS score was 6.36 ± 1.11 , suggesting that the overall research quality was acceptable.

Summary of GWASs and WES Study

In total, 72 variants were found to be significantly associated with NAFLD risk in the GWASs (Supplemental Table S1). Specifically, 52 variants were identified in adults, 15 variants were reported in children, and 6 variants were reported in both adults and children. Notably, *PNPLA3* rs738409 has been reported not only in adults, but also in

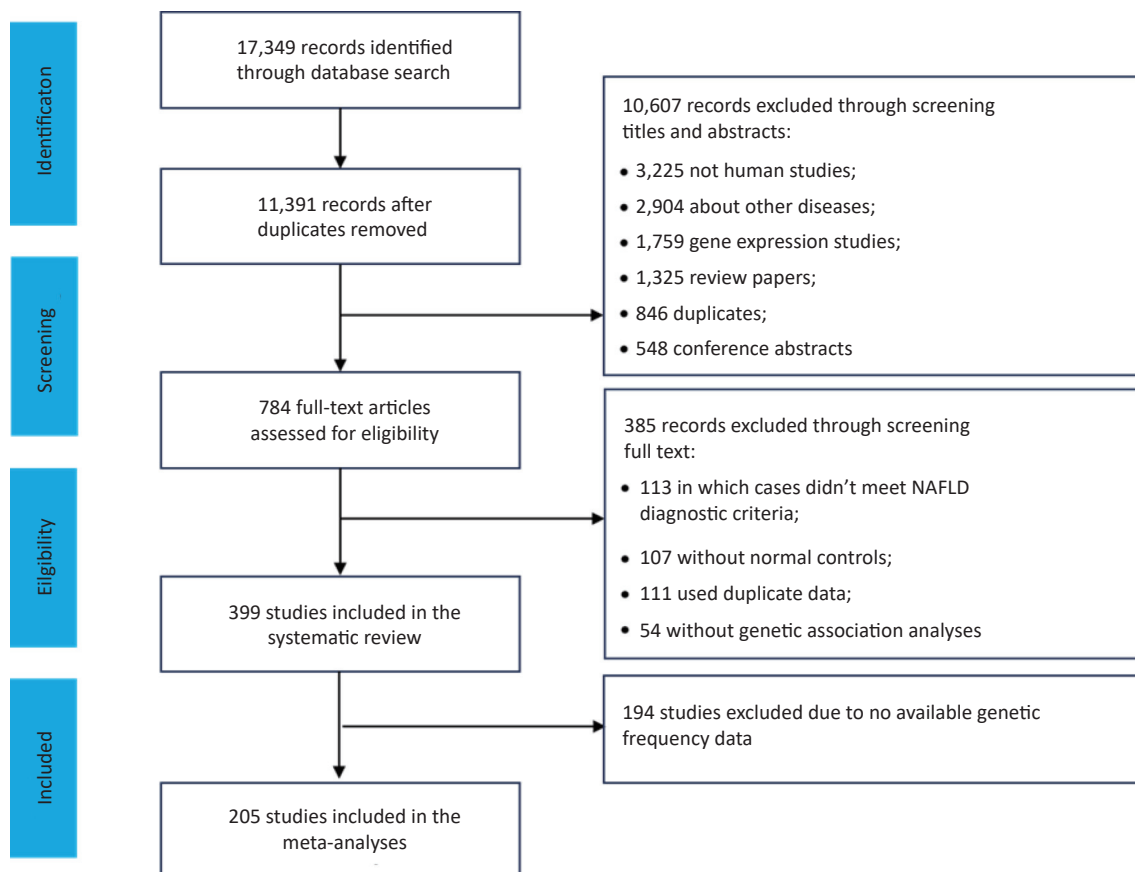


Figure 1. The flowchart for study selection.

mixed adults and children. In addition, two WES studies have shown that three variants of two genes (*PNPLA3*, and *PMPT*) are associated with NAFLD susceptibility in adults.

Summary of Candidate Gene Association Studies

Among the 465 genetic variants, common variants (416 variants in 157 genes) ($MAF \geq 5\%$) included synonymous single nucleotide polymorphisms (SNPs; $n = 20$), non-coding transcript SNPs ($n = 8$), intronic SNPs ($n = 208$), SNPs in 5' or 3' untranslated regions (UTRs; $n = 28$), missense variants ($n = 66$), upstream variants ($n = 46$), downstream variants ($n = 4$) and others ($n = 36$). Rare variants (49 variants in 34 genes) ($MAF < 5\%$) included synonymous variants ($n = 1$), non-coding transcript variants ($n = 2$), intronic variants ($n = 17$), variants in 5' or 3' UTRs ($n = 6$), missense variants ($n = 21$), and two upstream variants (Supplemental Table S3, available in www.besjournal.com). Based on the main function of each gene, the 173 genes were divided into eight categories: lipid synthesis and metabolism ($n = 49$), insulin resistance and glucose metabolism ($n = 10$), adipokines/adipokine receptors ($n = 6$), energy metabolism and obesity ($n = 6$), oxidative stress and antioxidants ($n = 22$), inflammatory and immune response ($n = 32$), liver fibrosis ($n = 11$) and others ($n = 37$), as shown in Supplemental Table S4 (available in www.besjournal.com).

Meta-Analysis of Associations Between Genetic Variations and NAFLD

As shown in Table 1, 25 variants of the 17 genes were included in the meta-analysis. The information extracted for each variant used in the meta-analysis is presented in Supplemental Table S5 (available in www.besjournal.com). These meta-analyses included a mean of 14 studies (range: 6–75) and 3281 participants (range: 1,784–42,903). 11 variants in 10 genes showed statistically significant associations with NAFLD ($P < 0.05$), including four genes related to lipid synthesis and metabolism (*MBOAT7*, *PEMT*, *PNPLA3*, *TM6SF2*), 1 gene related to insulin resistance and glucose metabolism (*GCKR*), 2 genes related to adipokines/adipokine receptors (*ADIPOQ*, *LEPR*), 2 genes related to oxidative stress and antioxidants (*HFE*, *MTHFR*), and 1 gene related to inflammatory and immune response (*TNF*). Moreover, all 11 variants in 10 genes showed positive associations with NAFLD, and the highest risk factor for NAFLD was *PNPLA3* rs738409 ($OR: 1.841$, 95% $CI: 1.691-2.004$). The cumulative

epidemiological evidence of a significant association was graded as strong for two variants in two genes (*HFE*, *TNF*), moderate for four variants in three genes (*TM6SF2*, *GCKR*, and *ADIPOQ*), and weak for five variants in five genes (*MBOAT7*, *PEMT*, *PNPLA3*, *LEPR*, and *MTHFR*), based on the Venice criteria.

Of the 25 variants in meta-analyses, seven variants had little or no inter-study heterogeneity ($I^2 < 25\%$), 7 variants showed moderate heterogeneity ($25\% \leq I^2 \leq 50\%$), and 11 variants showed high inter-study heterogeneity ($I^2 > 50\%$). The results of the dominant, recessive, heterozygous, and homozygous genetic models are shown in Supplemental Table S6 (available in www.besjournal.com). The allele contrast model identified more significant associations than the other models did.

All meta-analyses included a population of mixed ethnicities, except for *LEPR* rs1137100 and *LEPR* rs1137101, for which data were available only among Asian adults. Stratified meta-analyses according to ethnicity were conducted for the five variants of the four genes (Table 2). We found that *PNPLA3* rs738409 was associated with NAFLD in all ethnicities, but showed the highest OR value ($OR: 2.45$, 95% $CI: 2.04-2.95$) in Caucasians and the lowest OR value ($OR: 1.66$, 95% $CI: 1.51-1.83$) in Asians. The three variants showed significant associations in only one ethnic population in the stratified analysis. Despite significant associations with NAFLD in the total population, significant associations were observed for *GCKR* rs780094 in Asians, *GCKR* rs1260326 in Caucasians, and *MTHFR* rs1801133 in Asians.

Comparison between Previously Published Meta-Analysis Results and this Meta-Analysis

By 30 September, 2022, a total of 75 meta-analyses on NAFLD genetic associations were published involving 21 variants of 13 genes. The results of meta-analyses that were published most recently or included the largest number of studies were extracted for each variant (Table 3). Compared with previously published meta-analyses, meta-analyses for four variants in four genes were conducted for the first time in this study, and all four variants showed no association with NAFLD risk (*LYPLAL1* rs12137855, *PPARGC1A* rs8192678, *PPP1R3B* rs4240624, *NCAN* rs2228603). Among the variants that have been meta-analyzed in the past, consistent results were observed for 15 variants in 11 genes, where 10 variants in 9 genes were associated with increased risk of NAFLD (*TNF*

rs3615525, *ADIPOQ* rs266729, *GCKR* rs780094, *GCKR* rs1260326, *PNPLA3* rs738409, *MTHFR* rs1801133, *TM6SF2* rs58542926, *PEMT* rs7946, *LEPR* rs1137101, *HFE* rs1799945) and 5 variants in 4 genes (*TNF*

Table 1. Results of Random Effects Meta-Analyses Using Allelic Contrasts for Polymorphisms.

Gene and function	Variant	Alleles	MAF	Number assessed			Allelic contrasts		Heterogeneity			Venice criteria grade ^c	Cumulative evidence of association ^d
				Studies	Cases	Controls	OR (95% CI) ^a	P value	P value ^b	I ²			
Lipid synthesis and metabolism related genes													
<i>APOC3</i>	rs2854116	T vs C	0.55 (T)	11	3792	4601	0.99 (0.89-1.10)	0.826	0.004	63	ACC	+	
	rs2854117	C vs T	0.67 (C)	9	3538	3819	1.02 (0.95-1.10)	0.577	0.370	8	AAC	+	
<i>LYPLAL1</i> *	rs12137855	T vs C	0.20 (T)	13	4369	5292	0.98 (0.89-1.09)	0.753	0.326	12	AAC	+	
<i>MBOAT7</i>	rs641738	C vs T	0.57 (C)	12	4351	10,830	1.07 (1.00-1.14)	0.048	0.528	0	AAC	+	
<i>MTTP</i>	rs1800591	T vs G	0.15 (T)	11	1483	1490	0.89 (0.59-1.36)	0.592	< 0.001	88	ACC	+	
<i>PEMT</i>	rs7946	T vs C	0.70 (T)	10	1090	1390	1.51 (1.11-2.06)	0.008	< 0.001	73	ACA	+	
<i>PNPLA3</i>	rs738409	G vs C	0.26 (G)	75	18,193	24,710	1.84 (1.69-2.00)	< 0.001	< 0.001	85	ACA	+	
<i>PPARG</i>	rs1801282	G vs C	0.11 (G)	9	2108	2740	0.88 (0.70-1.10)	0.260	0.084	43	ABC	+	
<i>PPARGC1A</i> *	rs8192678	T vs C	0.33 (T)	6	726	1058	1.05 (0.78-1.41)	0.735	0.011	66	ACC	+	
<i>TM6SF2</i>	rs58542926	T vs C	0.07 (T)	24	5499	12,677	1.69 (1.47-1.93)	< 0.001	0.068	33	ABA	++	
Insulin resistance and glucose metabolism related genes													
<i>GCKR</i>	rs780094	T vs C	0.40 (T)	23	6401	9983	1.18 (1.12-1.26)	< 0.001	0.030	39	ABA	++	
	rs1260326	T vs C	0.40 (T)	9	1655	2527	1.48 (1.67-1.87)	0.001	< 0.001	46	ABA	++	
<i>PPP1R3B</i> *	rs4240624	G vs A	0.11(G)	7	2362	3292	0.88 (0.68-1.14)	0.339	0.367	8	AAC	+	
Adipokines/adipokine receptors related genes													
<i>ADIPOQ</i>	rs1501299	T vs G	0.27 (T)	13	2261	2190	0.97 (0.74-1.29)	0.849	< 0.001	88	ACC	+	
	rs266729	G vs C	0.23 (G)	8	1875	1466	1.61 (1.36-1.91)	< 0.001	0.142	40	ABA	++	
	rs2241766	G vs T	0.10 (G)	13	2187	2072	1.09 (0.92-1.28)	0.335	0.012	54	ACC	+	
<i>LEPR</i>	rs1137100	G vs A	0.27 (G)	7	1382	1304	1.02 (0.83-1.26)	0.863	0.131	39	ABC	+	
	rs1137101	G vs A	0.46 (A)	6	1591	1535	1.82 (1.41-2.54)	< 0.001	0.019	63	ACA	+	
Oxidative stress and antioxidants related genes													
<i>HFE</i>	rs1800562	A vs G	0.05 (A)	15	2261	5508	1.83 (0.99-3.40)	0.056	< 0.001	63	ACA	+	
	rs1799945	G vs C	0.14 (G)	14	1993	2475	1.24 (1.04-1.48)	0.019	0.192	24	AAA	+++	
<i>MTHFR</i>	rs1801131	C vs A	0.30 (C)	7	2122	1157	1.24 (0.93-1.65)	0.141	< 0.001	76	ACA	+	
	rs1801133	T vs C	0.34 (T)	9	2303	1399	1.30 (1.06-1.59)	0.012	0.006	63	ACA	+	
Inflammatory and immune response related genes													
<i>TNF</i>	rs3615525	A vs G	0.05 (A)	14	2055	1594	1.82 (1.42-2.34)	< 0.001	0.219	23	AAA	+++	
	rs1800629	A vs G	0.15 (A)	13	2176	1789	1.29 (0.99-1.69)	0.064	0.115	34	ABA	++	
Other functional genes													
<i>NCAN</i> *	rs2228603	T vs C	0.07 (T)	11	4251	6105	0.97 (0.86-1.08)	0.544	0.712	0	AAC	+	

Note. OR: odds ratio; CI: confidence interval; G: guanine; A, adenine; C: cytosine; T: thymine; MAF: minor-allele frequency. * Genes and loci that have not been meta-analyzed in published literature; ^a Summary ORs are based on random-effects allelic contrasts comparing minor and major alleles (based on frequencies in the control samples); ^b Based on the Q statistic across crude ORs calculated for each study; ^c Degree of 'epidemiological credibility' based on the interim Venice guidelines (A, strong; B, modest; C, weak); ^d Cumulative epidemiological evidence as graded by Venice criteria as strong (+++), moderate (++), or weak (+) for association with NAFLD risk.

rs1800629, *PPARG* rs1801282, *APOC3* rs2854116, *APOC3* rs2854117, *LEPR* rs1137100) showed no associations with NAFLD. Five variants in four genes (*ADIPOQ* rs1501299, *ADIPOQ* rs2241766, *MTPP* rs1800591, *MTHFR* rs1801131, *HFE* rs1800562) showed significant associations in previous meta-analyses but showed insignificant associations in this study. In contrast, *MBOAT7* rs641738 was not associated with NAFLD in previous meta-analyses but was significantly associated with NAFLD in this study.

DISCUSSION

This study comprehensively reviewed the genetic

association with NAFLD susceptibility by analyzing 381 candidate gene studies, 16 GWASs, and two WESs. 72 and 465 variants of over 100 genes related to multiple functional pathways were identified in the GWASs and candidate gene studies, respectively. We conducted meta-analyses of 25 variants and found significant associations with NAFLD risk for 11 variants, including six variants that showed moderate to strong cumulative epidemiological evidence for a true association. Additionally, meta-analyses provided convincing evidence of no association between NAFLD risk and 14 variants in 11 genes.

Our study identified 4 SNPs in 4 genes related to lipid synthesis and metabolism that were

Table 2. Results of Random Effects Meta-Analyses of Alleles Using Allelic Contrasts for Polymorphisms Stratified by Ethnicity.

Gene and function	Variant	Alleles	Subgroup	Number assessed			Allelic contrasts		Heterogeneity			Venice criteria grade ^c	Cumulative evidence of association ^d
				Studies	Cases	Controls	OR (95% CI) ^a	P value	P value ^b	I ²			
Lipid synthesis and metabolism related genes													
<i>MBOAT7</i>	rs641738	C vs T	Total	12	4351	10,830	1.07 (1.00-1.14)	0.048	0.528	0	AAC	+	
			Caucasians	7	2464	2166	1.07 (0.98-1.16)	0.144	0.448	0	AAC	+	
			Asians	5	1887	8664	1.07 (0.97-1.19)	0.188	0.374	6	AAC	+	
<i>PNPLA3</i>	rs738409	G vs C	Total	75	18,193	24,710	1.84 (1.69-2.00)	< 0.001	< 0.001	85	ACA	+	
			Caucasians	21	4144	3327	2.45 (2.04-2.95)	< 0.001	< 0.001	77	ACA	+	
			Asians	45	12,792	20,320	1.66 (1.51-1.83)	< 0.001	< 0.001	86	ACA	+	
			Others	4	742	299	1.89 (1.52-2.37)	< 0.001	0.961	0	AAA	+++	
Insulin resistance and glucose metabolism related genes													
<i>GCKR</i>	rs780094	T vs C	Total	23	6401	9983	1.18 (1.12-1.26)	< 0.001	0.030	39	ABA	++	
			Asians	21	5603	9456	1.17 (1.10-1.26)	< 0.001	0.040	38	ABA	++	
			Caucasians	2	798	527	1.34 (0.91-1.98)	0.142	0.065	71	ABC	+	
<i>GCKR</i>	rs1260326	T vs C	Total	9	1655	2527	1.48 (1.67-1.87)	0.001	< 0.001	46	ABA	+++	
			Asians	4	1023	992	1.61 (0.99-2.62)	0.057	< 0.001	93	ACA	+	
			Caucasians	3	393	552	1.42 (1.17-1.71)	< 0.001	0.776	0	BAA	++	
			Others	2	239	983	1.33 (0.74-2.36)	0.338	0.038	77	ACA	+	
Oxidative stress and antioxidants related genes													
<i>MTHFR</i>	rs1801133	T vs C	Total	9	2303	1399	1.30 (1.06-1.59)	0.012	0.006	63	ACA	+	
			Caucasians	4	1663	714	1.20 (0.85-1.68)	0.296	0.013	72	ACA	+	
			Asians	3	471	506	1.51 (1.07-2.12)	0.019	0.077	61	BCA	+	
			Hispanics	2	169	179	1.28 (0.63-2.58)	0.499	0.058	72	BCA	+	

Note. OR: odds ratio; G: guanine; C: cytosine; T: thymine. ^a Summary ORs are based on random-effects allelic contrasts comparing minor and major alleles (based on frequencies in the control samples); ^b Based on the *Q* statistic across crude ORs calculated for each study; ^c Degree of 'epidemiological credibility' based on the interim Venice guidelines (A, strong; B, modest; C, weak); ^d Cumulative epidemiological evidence as graded by Venice criteria as strong (+++), moderate (++), or weak (+) for association with NAFLD risk.

Table 3. Previously published meta-analyses results compared to meta-analyses in this study.

Gene	Study	Polymorphism	Prior meta sample		Model	Published meta OR		Our new meta		Model	New meta OR (95% CI)	Het. Meta
			size: cases; controls (number of samples)	size: cases; controls (number of samples)		Meta	Het. Meta	sample size: cases; controls (number of samples)	Het. Meta			
TNF	Wang et al. 2011	rs3615525	771; 787 (7)	GA/AA vs GG		2.06 (1.58-2.69) ^a	0.160	2055; 1594 (14)	A vs G		1.82 (1.42-2.34)	0.219
		rs1800629	837; 990 (8)	GA/AA vs GG		1.08 (0.82-1.42) ^a	0.860	2176; 1789 (13)	A vs G		1.29 (0.99-1.69)	0.115
PPARG	Zhang et al. 2015	rs1801282	1697; 2427 (8)	GC/GG vs CC		0.93 (0.63-1.38)	<0.001	2108; 2740 (9)	G vs C		0.88 (0.70-1.10)	0.084
	Wang et al. 2014	rs266729	876; 989 (7)	GG/GC vs CC		1.52 (1.10-2.09) ^a	0.280	1875; 1466 (8)	G vs C		1.61 (1.36-1.91)	0.142
ADIPOQ	Wang et al. 2016	rs1501299	1117; 1555 (10)	G vs T		1.27 (1.10-1.48) ^a	0.533	2261; 2190 (13)	T vs G		0.97 (0.74-1.29)	<0.001
		rs2241766	1117; 1555 (10)	T vs G		1.33 (1.12-1.58) ^a	0.151	2187; 2072 (13)	G vs T		1.09 (0.92-1.28)	0.012
APOC3	Li et al. 2017	rs2854116	2111; 1866 (9)	C vs T		1.39 (0.96-2.02) ^a	0.001	3792; 4601 (11)	T vs C		0.99 (0.89-1.10)	0.004
		rs2854117	2111; 1866 (9)	T vs C		1.05 (0.92-1.19) ^a	0.840	3538; 3819 (9)	C vs T		1.02 (0.95-1.10)	0.370
GCKR	Li et al. 2021	rs780094	5115; 11,812 (20)	T vs C		1.20 (1.11-1.29)	0.020	6401; 9983 (23)	T vs C		1.17 (1.10-1.24)	<0.001
		rs1260326	2238; 8995 (9)	T vs C		1.32 (1.22-1.42)	0.560	1655; 2527 (9)	T vs C		1.27 (1.14-1.42)	<0.001
MTHFR	Sun et al. 2016	rs1801131	364; 611 (5)	C vs A		1.53 (1.13-2.07)	0.001	2122; 1157 (7)	C vs A		1.24 (0.93-1.65)	<0.001
		rs1801133	737; 1160 (8)	TT vs TC/CC		1.42 (1.07-1.88)	0.160	2303; 1399 (9)	T vs C		1.30 (1.06-1.59)	0.006
MBOAT7	Xia et al. 2019	rs641738	2560; 8738 (5)	C vs T		0.99 (0.93-1.05) ^a	-	4351; 10,830 (12)	C vs T		1.07 (1.00-1.14)	0.528
	Chen et al. 2019	rs58542926	3075; 3000 (13)	Unknown		0.55 (0.48-0.63)	-	5499; 12,677 (24)	T vs C		1.69 (1.47-1.93)	0.068
PEMT	Tan et al. 2016	rs7946	792; 2722 (6)	TT/TC vs CC		1.62 (1.10-2.39)	-	1090; 1390 (10)	T vs C		1.51 (1.11-2.06)	<0.001
		rs1137100	1111; 1132 (6)	A vs G		1.01 (0.87-1.18)	0.110	1382; 1304 (7)	G vs A		1.02 (0.83-1.26)	0.131
LEPR	Pan et al. 2018	rs1137101	1298; 1348 (5)	A vs G		0.57 (0.50-0.65)	0.140	1591; 1535 (6)	G vs A		1.82 (1.41-2.54)	0.019
		rs1800562	1846; 7037 (11)	A vs G		1.95 (1.16-3.28)	<0.001	2261; 5508 (15)	A vs G		1.83 (0.99-3.40)	<0.001
HFE	Ye et al. 2016	rs1799945	3945; 12,332 (16)	G vs C		1.21 (1.07-1.38) ^a	0.338	1993; 2475 (14)	G vs C		1.24 (1.04-1.48)	0.192

Note. ^a Fixed effects model was used in prior meta-analysis; P-value is not reported.

significantly associated with NAFLD risk. An association with *TM6SF2* rs58542926 has been identified in several GWASs and a meta-analysis by Chen et al. (2019)^[24]. The SNP *TM6SF2* rs58542926 leads to a reduction in the expression and activity of *TM6SF2*, which normally promotes triglyceride secretion from hepatocytes^[10,25]. The *PNPLA3* rs738409 SNP causes a missense coding of methionine instead of isoleucine, leading to reduced lipidation of Apolipoprotein B100 and promotion of hepatic fat accumulation^[26,27], and has been reported as a genetic risk factor for NAFLD in a large number of studies, including several GWASs^[7-9] and previous meta-analyses^[28,29]. *MBOAT7* rs641738 was found to have borderline significance in this study, whereas a previous meta-analysis by Xia et al. (2019)^[30] reported insignificant associations. The SNP rs641738 is believed to affect the acyl chain composition and remodeling of phospholipids in membranes^[31]. The *PEMT* rs7946 variant leads to an amino acid substitution and reduced *PEMT* activity, causing the accumulation of triacylglycerols in the liver^[32]. A significant association between *PEMT* rs7946 polymorphism and NAFLD has only been reported in Asians in a meta-analysis conducted by Tan et al. (2016)^[33], and this association was confirmed in a mixed population in this study, although only weak cumulative epidemiological evidence was found.

Among insulin resistance and glucose metabolism-related genes, this study found moderate evidence of an association between two *GCKR* variants (rs780094 and rs1260326) and NAFLD risk, supported by previous GWASs and meta-analyses^[34,35]. *GCKR* encodes a protein that regulates glucokinase activity^[36], and the polymorphism rs1260326 leads to increased glucokinase activity and promotes hepatic glucose metabolism and lipid synthesis^[37]. The intronic SNP rs780094 has been linked to fasting serum triacylglycerol, insulin, and type 2 diabetes risk^[38] and is in strong linkage disequilibrium with rs1260326, resulting in reduced regulation of glucokinase activity by *GCKR*^[39]. In addition, an epigenetic study suggested that rs780094 functions as a transcriptional enhancer of *GCKR* expression^[40].

Two genes related to adipokines or adipokine receptors were identified in this study (*ADIPOQ*, *LEPR*), and moderate evidence was observed for *ADIPOQ* rs266729. Adiponectin, the protein product of the *ADIPOQ* gene, is an anti-inflammatory adipokine that functions as an insulin sensitizer and regulates energy homeostasis and glucose and lipid

metabolism^[41]. A GWAS found that the *ADIPOQ* gene was the major contributor to plasma adiponectin, accounting for 6.7% of its variation^[42]. The rs266729 SNP in the *ADIPOQ* promoter region regulates adiponectin levels and promoter activity^[43].

We also identified two genes related to oxidative stress and antioxidants (*HFE*, *MTHFR*) that were associated with NAFLD. *HFE* regulates cellular iron uptake^[44] and the rs11799945 polymorphism is significantly linked to NAFLD with strong cumulative epidemiological evidence, which is consistent with a previous meta-analysis^[14]. Additionally, *HFE* rs1800562 was only observed among Caucasians and was not detected in studies on Asian populations. Consistent with this, the online dbSNP database showed that the percentages of mutated A alleles reported in Caucasians and Asians were 0.059 and 0.0003, respectively. *MTHFR* rs1801133 showed a significant association with NAFLD; however, a meta-analysis of nine studies showed moderate heterogeneity. Consistent results for *MTHFR* rs1801133 polymorphism were reported in a meta-analysis by Sun et al. (2016)^[45], although they also identified a significant association with *MTHFR* rs1801131, which was not significant in this study. Rs1801133 is a common missense variant (C677T) that results in a less active *MTHFR* enzyme^[46,47] and is associated with a higher concentration of homocysteine, which may induce oxidative stress and endoplasmic reticulum stress^[48].

We found that *TNF* rs361525 had strong cumulative evidence of an association with NAFLD, whereas *TNF* rs1800629 showed no significant association, consistent with a previous meta-analysis by Wang et al. (2012)^[49]. *TNF* gene encodes tumor necrosis factor alpha (*TNF α*), a pro-inflammatory cytokine involved in the development of NAFLD^[50]. However, the role of *TNF* rs361525 remains elusive, and mixed findings were reported regarding rs361525 and the level of *TNF α* in blood^[51,52]. Further studies are required to elucidate the potential mechanisms for *TNF* polymorphisms in the development of NAFLD.

This study compared the results to those of previous meta-analyses and found consistency in 14 variants in 10 genes, and inconsistency in six variants in five genes. These differences may be attributed to the updated number of included studies and differences in the inclusion and exclusion criteria. For example, some previous studies contained data that did not conform to the Hardy-Weinberg equilibrium or included samples with other chronic liver diseases. In addition, the inconsistent results

may also be a result of different selections of genetic models. In this meta-analysis, we used the results based on allele contrast models for comparison with previous studies.

To the best of our knowledge, this is the first study to comprehensively review studies analyzing genetic susceptibility in NAFLD and to extract data from GWASs, WES studies, and candidate gene studies. Potential susceptibility genes were categorized into eight categories based on their genetic functions, which may have facilitated the interpretation of the findings. Our study has certain limitations. First, although a thorough literature search was conducted using several strategies, it is likely that some publications were overlooked. Second, studies not published in English and publications without resolvable genotype counts were not included, which may have resulted in a publication bias. However, these studies probably represent a small percentage of all publications that investigated NAFLD-associated variants, and we did not detect significant publication bias in most meta-analyses. Third, the meta-analyses were conducted only for variants with a minimum of five data sources. Fourth, the use of genotype counts and crude estimates of effects precludes more sophisticated analyses, including adjusted estimates of association and gene-gene and gene-environment interactions, for which raw genotype data are required. Fifth, although we conducted a stratified analysis by ethnicity, we were unable to examine the potential sources of heterogeneity, which were limited by the number of studies available. Sixth, there was heterogeneity between the studies on the diagnostic methods used to define NAFLD. Finally, most of the positive variants in this study did not reach very high levels of statistical significance, and those with modest *P* values should be considered cautiously. Issues such as multiple testing, linkage disequilibrium among associated variants, and undetected publications or other reporting biases may mask the observed association.

In conclusion, this systematic review and meta-analysis provides a comprehensive summary of the genetic susceptibility to NAFLD. Recent GWASs and WES studies have identified 72 variants associated with the risk of NAFLD. Meta-analyses identified 11 genetic variants with strong or moderate evidence of association, and further study of these variants is required. We also identified 14 variants in 11 genes that showed no evidence of association with NAFLD risk. These findings provide valuable information for future studies to assess genetic factors associated

with the risk of NAFLD.

AUTHOR CONTRIBUTIONS

LI Ya Mei and LUO Jia You designed the study; LI Ya Mei, XIAO Xiang, WANG Jie, and YU Hai Bin conducted literature searches and data extraction; XIAO Xiang, LI Ya Mei, WANG Jie, LIU Yi Xu, and PAN Xiong Feng conducted statistical analyses; LI Ya Mei, XIAO Xiang, and LUO Mi Yang wrote the manuscript; LUO Mi Yang, LUO Jia You, WANG Jie, LIU Yi Xu, PAN Xiong Feng, and YU Hai Bin reviewed and edited the manuscript. All authors critically reviewed the manuscript and have approved of the final draft of the manuscript.

SUPPLEMENTAL MATERIALS

Appendix A: Detailed search strategy.

Web of science:

TS=("non-alcoholic fatty liver disease" OR "nonalcoholic fatty liver disease" OR NAFLD OR "non-alcoholic fatty liver" OR "nonalcoholic fatty liver" OR NAFL OR "non-alcoholic steatohepatitis" OR "nonalcoholic steatohepatitis" OR NASH OR "hepatic steatosis") AND TS=(SNP* OR polymorphism* OR variant* OR variation* OR mutation*) AND language: (English OR Chinese) The index=SCI-EXPANDED, CPCI-S, ESCI timescale=1980-2022

Pubmed:

((("non-alcoholic fatty liver disease" OR "nonalcoholic fatty liver disease" OR NAFLD OR "non-alcoholic fatty liver" OR "nonalcoholic fatty liver" OR NAFL OR "non-alcoholic steatohepatitis" OR "nonalcoholic steatohepatitis" OR NASH OR "hepatic steatosis") AND (SNP* OR polymorphism* OR variant* OR variation* OR mutation*)) AND ((humans) AND (chinese OR english) AND (1980:2022))

Embase: ('non-alcoholic fatty liver disease':ab,ti OR 'nonalcoholic fatty liver disease':ab,ti OR nafld:ab,ti OR 'non-alcoholic fatty liver':ab,ti OR 'nonalcoholic fatty liver':ab,ti OR naf:ab,ti OR 'non-alcoholic steatohepatitis':ab,ti OR 'nonalcoholic steatohepatitis':ab,ti OR nash:ab,ti OR 'hepatic steatosis':ab,ti) AND (snp*:ab,ti OR polymorphism*:ab,tiORvariant*:ab,tiORvariation*:ab,tiORmutation*:ab,ti) AND (/lim OR /lim) AND /lim AND /lim AND /py

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