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REVIEW ARTICLE

Association of Tumor Necrosis Factor-α gene Polymorphisms with Non-alcoholic Fatty Liver Disease: Systematic Review with Meta-Analysis

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Key words:
Non-alcoholic fatty liver disease, tumor necrosis factor-alpha, polymorphism, meta-analysis, systematic review

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ABSTRACT

Background: *Nonalcoholic fatty liver disease (NAFLD) is one of the most prevalent liver diseases that is affected by various environmental and genetic factors. The association between tumor necrosis factor-alpha (TNF-α) gene polymorphism in regions -308G/A (rs1800629) and -238G/A (rs361525) and susceptibility to NAFLD is controversial.* ***Objective****: This meta-analysis evaluated the association between different candidate TNF-α polymorphisms and NAFLD.* ***Methodology:*** *A systematic search was conducted on PubMed, Cochrane Library, and Egyptian Knowledge Bank accessing the following databases: Scopus, Web of science, EBSCO and Ovid, and EMBASE to retrieve all relevant studies published until March 29, 2023. Based on predetermined selection criteria, all eligible studies were included in the meta-analysis. Odds ratios (ORs) with 95% confidence intervals (CIs) were estimated.* ***Results:*** *The systematic search revealed that five TNF-alpha polymorphisms were studied concerning NAFLD risk: TNF-α -1031(rs1799964), -857(rs1799724), -308(rs1800629), -238(rs361525), and -863(rs1800630), with the last three polymorphisms were eligible for meta-analysis. Eleven studies with 1155 NAFLD cases and 1364 controls demonstrated the significant association between rs1800629 polymorphism and NAFLD under both the dominant model [OR = 1.27, 95% CI = 1.01–1.59, P = 0.04] and allelic contrast [OR = 1.26, 95% CI = 1.03–1.54, P = 0.02], with no significant association under heterozygous comparison. Considering the rs361525 polymorphism, meta-analysis including nine studies with 904 cases and 848 controls suggested significant association under each of the dominant model [OR = 1.76, 95% CI = 1.14–2.71, P =0.01], heterozygous model [OR = 1.77, 95% CI = 1.14–2.74, P = 0.01], and the allelic model [OR = 1.66, 95% CI = 1.31–2.44, P = 0.01]. However, no association was found between rs1800630 polymorphism and NAFLD risk.* ***Conclusion:*** *This meta-analysis suggested that TNF-α gene polymorphisms rs361525 and rs1800629, but not rs1800630, might be a risk factor for NAFLD.*

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has become the most common liver disease, with an estimated 30% prevalence globally[1]. It includes a spectrum ranging from simple steatosis (non-alcoholic fatty liver or NAFL), non-alcoholic steatohepatitis (NASH), NASH-related cirrhosis, and hepatocellular carcinoma (HCC)[2].

Various risk factors have been associated with NAFLD and NASH, such as insulin resistance (IR) 3, obesity, type 2 diabetes mellitus, hypercholesterolemia, drugs, toxins[4,5] mitochondrial dysfunction, and oxidative stress[6].

Additionally, different gene polymorphisms have been proposed to increase susceptibility to NAFLD and enhance progression to NASH, particularly genes involved in lipid metabolism and steatosis, the insulin signaling pathway, reactive oxygen species (ROS) formation and degradation, cytokines and endotoxin receptors, and profibrogenic mediators [7,8].

Tumor necrosis factor alpha (TNF-α) is an important cytokine that has been suggested to play a critical role in the pathogenesis of NAFLD[9].

In addition, TNF-α may potentiate hepatic IR through affecting insulin receptor substrate (IRS)-1 and insulin receptor kinase (IRK) in the insulin signal transduction pathway and promote decomposition of adipocytes and release of free fatty acids [10]

A Meta-analysis included fifty-six studies, reported higher levels of circulating TNF-α in NAFLD patients compared to controls which is also associated with severity of NAFLD [11].

Studies have shown that allele polymorphisms at positions -308 (rs1800629) or -238(rs361525) of the TNF-α gene promoter may affect TNF-α expression and

may induce IR [12]. Moreover, these polymorphisms have been involved in the pathogenesis and progression of NASH [13].

Several genetic association studies examined the association between TNF-α Single Nucleotide Polymorphisms (SNPs) and susceptibility to NAFLD[12,14–25].

Some studies suggested that TNF-α polymorphism -238 (rs361525) but not -308 (rs1800629) is significantly associated to increased NAFLD risk [22,23,25]. On the other hand, other studies suggested that -308(rs1800629) SNP has significant relation to susceptibility to NAFLD [19,26]. However, other studies have shown controversial results[14,15].

In Japan, Tokushige et al. investigated 5 TNF alpha polymorphisms at positions: -1031, -863, - 857, -308, and -238, concluding that there were no significant association between any of studied TNF alpha SNPs and increase risk to NAFLD [14].

Meta-analysis is a statistical analysis that allows integration of the results of independent studies. Moreover, it provides a precise estimate of the effect size and increasing statistical power, which is particularly important for primary studies with low statistical power due to their small sample size. Meta-analysis examines the inter-study heterogeneity, solving a frequent problem of variation in the findings across different genetic association studies. Therefore, meta-analysis is potentially a powerful method for quantitatively evaluating the effects of candidate genes[27, 28].

A previous meta-analysis concluded that gene polymorphism at position -238, but not -308, was significantly associated with susceptibility to NAFLD[29].

After more than a decade with the publication of new relevant studies [18–20, 26] the association between TNF alpha gene polymorphisms needed reevaluation. This study aimed to conduct a systematic review with meta-analysis to quantitatively assess the association between any candidate TNF-α gene polymorphisms and the susceptibility of NAFLD.

METHODOLOGY

Methods of this analysis were based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement 2020 [30].

Search strategy:

We conducted searches on PubMed, Cochrane Library, and Egyptian Knowledge Bank accessing the following databases: Scopus, Web of Science, EBSCO and Ovid, and EMBASE. The last retrieval was performed on March 29, 2023, assembling the following search strategy (("Non-alcoholic Fatty Liver Disease" OR "Non alcoholic Fatty Liver Disease" OR "NAFLD" OR "Nonalcoholic Fatty Liver Disease" OR "Nonalcoholic Fatty Liver" OR "Nonalcoholic steatohepatitis" OR NASH) AND (("Tumor Necrosis Factor-alpha" OR "Tumor Necrosis Factor alpha" or "TNFalpha" OR "TNF-alpha" or "Tumor Necrosis Factor-α" OR "TNF-α") AND ("Single Nucleotide Polymorphism*" OR SNPs OR "Genetic varia*" OR "Gene varia*OR Gene polymorphism*OR Genetic polymorphism*" OR " Gene muta*"))). Other potential eligible studies were searched manually for fulfilling our criteria through the reference lists from retrieved articles.

Study Selection:

Full-text screening was independently performed by 2 reviewers according to the following eligibility criteria. The main inclusion criteria were: a) evaluation of association between NAFLD and TNF-alpha polymorphisms; b) case‑control studies that involved adult human subjects (≥18 years old); c) the full text was available and reporting genotype frequencies in cases and controls, or sufficient data to estimate odds ratios (ORs) and 95% confidence intervals (CIs); d) studies published in the English language. The major exclusion criteria of studies were a) repeated records; b) review articles, editorial comments, case reports and animal studies; c) no information on genotype frequency, d) investigated another (gene) polymorphisms other than TNF-α, e) studied population were children and adolescents.

Data extraction:

Two researchers extracted the data independently. We extracted the following data: 1) first author's name, year of publication; 2) sample size of each group; 3) participants characteristics in cases and controls (ethnicity or geographic location of studied population, source of controls); 4) genotyping method; 5) NAFLD diagnosis method; 6) genotype distributions and Hardy Weinberg equilibrium test in controls.

Quality score assessment:

The Newcastle-Ottawa Scale (NOS) [31] was used to evaluate the methodological quality of involved studies on 3 main domains: cases selection, comparison of populations, and ascertainment of exposure to risks. The NOS ranges between zero (worst) and 9 stars (best) and NOS scores of studies ≥7 were considered of high-quality.

Statistical analysis:

Hardy-Weinberg equilibrium (HWE) of the genotype distributions of the included TNF-alpha polymorphisms; rs1800629, rs361525, and rs1800630 among controls was tested using an exact test ($P>0.05$)[32, 33].

The pooled ORs and the corresponding 95% CIs was computed to estimate the correlation between each of rs1800629, rs361525, and rs1800630 polymorphisms and NAFLD risk. The significance of pooled ORs was determined using the Z test, with $P < 0.05$ defined as significant. Moreover, a chi-square-based Q-test was applied to assess heterogeneity among studies. The

random-effect model (DerSimonian-Laird method) was applied when a probability value of P<0.10 for the Q test [34]. Otherwise, the fixed effect model (Mantel-Haenszel method) was adopted [35].

Genetic models:

Generally, pooled effect of correlation between the candidate SNP and the outcome i.e. NAFLD susceptibility is assessed in 5 genetic models: allele model, dominant model, heterozygote model, homozygote model, and recessive model [36,37]. In the current study, out of five suggested models, only the first three inheritance models were applicable, as the frequency of the AA genotype were zero in most of the included studies.

Subgroup Analysis: Stratified analyses were further conducted based on ethnicity (Asian and Caucasian), and Hardy-Weinberg equilibrium achievement among controls.

Publication bias and sensitivity analysis: Publication bias was assessed using funnel plot and Egger's test with P values less than 0.05 were considered as significant [38]. Publication bias was assessed only for meta-analyses included 10 or more studies [39]. A sensitivity analysis that assesses the impact of excluding certain studies, one by one, was also carried out [40]. The meta-analysis was repeated after excluding studies with the controls not in HWE [41, 42].

Software used: In the current meta-analysis, Review Manager 5.4.1 (Cochrane Collaboration) was used to perform Forest plots, while the Egger linear regression method was conducted using R software 4.3.2 version to assess the publication bias.

RESULTS

Eligible studies:

Based on our search strategy, a total of 416 potentially relevant studies were identified by initial retrieval from the databases, and 340 records were selected after removing duplicates. Among them, 314 irrelevant records were excluded by titles and abstract screening.

Out of 26 candidate studies, 2 studies excluded due to failure to retrieve their full text. The remaining 24 studies manuscripts were evaluated for eligibility, of which 13 studies were further excluded either for investigating other gene polymorphisms, not case-control studies, or inappropriate outcome and rational. Additionally, two extra studies were included form previous systematic review [24,25]. A total of 13 eligible studies were included in our meta-analyses, with 11 papers investigated -308(rs1800629) SNP, 9 papers studied -238 (rs361525) SNP, and only 2 papers reported data about the -863 (rs1800630) SNP. The PRISMA flow chart of the literature search and selection process is presented in Figure1.

Baseline characteristics:

The included studies conducted across different 11 countries throughout period between 2002 and 2022. Asian populations were studied in 5 studies while the remaining 8 article were conducted on Caucasians. In three studies, controls were community based [22,23], while in 10 studies they were hospital based [12,14–21,24,25].

For NAFLD diagnosis, six studies used liver biopsy for all patients to diagnose NAFLD [14–16,20,21,24], while the remaining studies either used ultrasonography alone [12,17–19,23]or combined it with liver biopsy for some patients [22,25].

Polymerase chain reaction‑restriction fragment length polymorphism (PCR‑RFLP) was the genotyping method in seven studies [18,20–25], while other included studies used various genotyping methods [12,14–17,19].

Using New Castle Ottawa scale (NOS) to assess the quality of the included studies, 11 studies scored ≥7[12,14–23], with 6 score for the remaining 2 studies [24,25]. Thus, the overall quality of the included studies was adequate.

Among all included studies, the distribution of genotypes in controls was consistent with Hardy-Weinberg equilibrium expect for two studies for rs1800629 SNP [19,26], and one study for rs1800630 SNP [14]. Characteristics of the involved studies are detailed in Table 1 and 2.

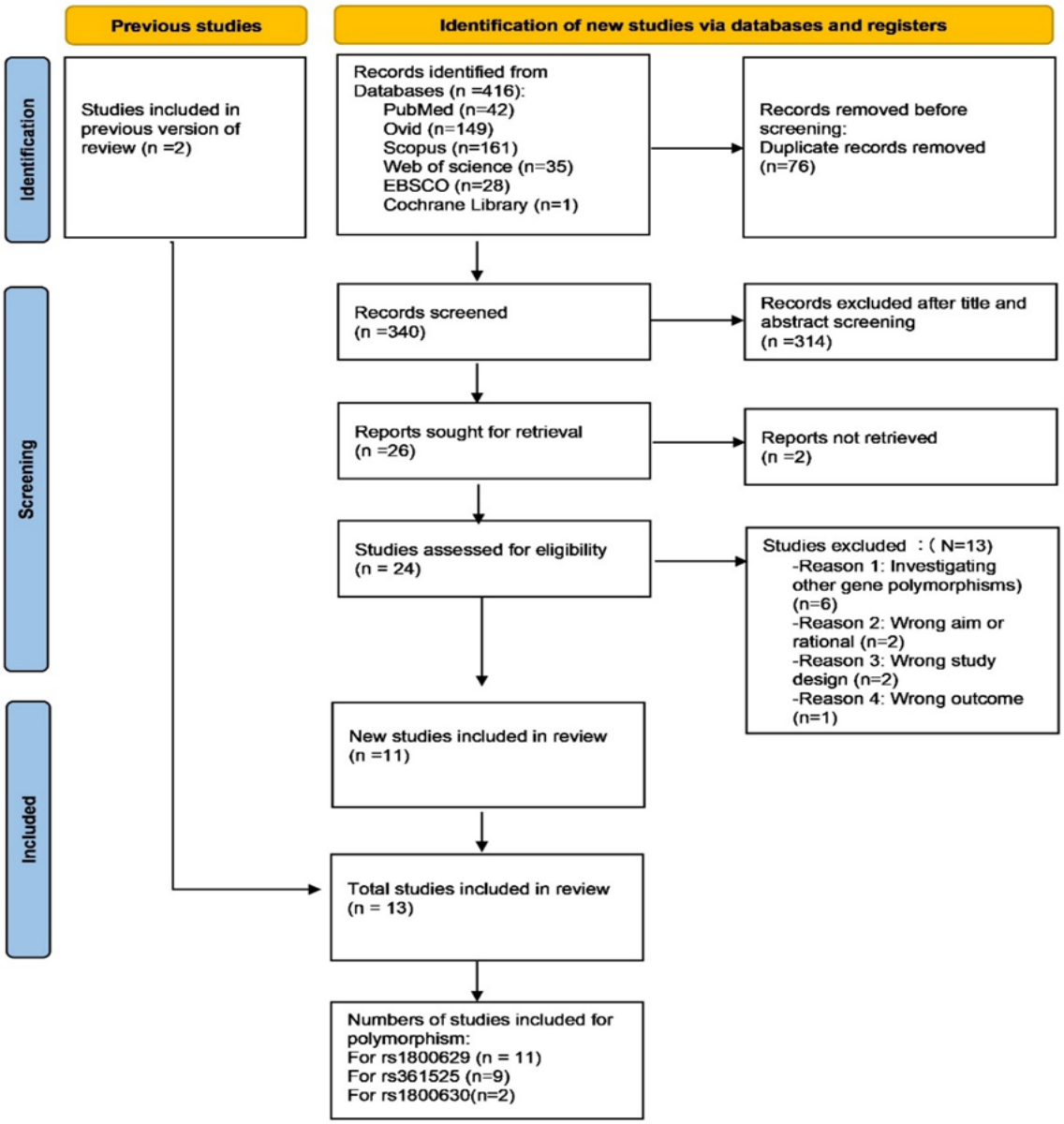

Figure 1. PRISMA flow diagram of the study selection process.
PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-analyses

Table 1: Characteristics of the involved studies in the association between Tumor necrosis factor alpha (TNF-α) gene polymorphisms and susceptibility to NAFLD

First Author	Year	Country	Race	Studied TNF-α SNPs	Cases (NO.)	Control (NO.)	Source of controls	NAFLD diagnosis	Genotyping method	NOS
Valenti L [25]	2002	Italy	Caucasians	-308(rs1800629), -238(rs361525)	99	172	HB	US; LB	PCR-RFLP	6
Tokushige K [14]	2007	Japan	Asians	-1031(rs1799964), -863(rs1800630), -857(rs1799724), -308(rs1800629), -238(rs361525)	102	100	HB	LB	Direct sequencing	7
Wong VW [15]	2008	China	Asians	-863(rs1800630), -308(rs1800629), -238(rs361525)	79	40	HB	LB	TaqMan	7
Hu ZW [22]	2009	China	Asians	-308 (rs1800629), -238(rs361525)	189	138	PB	US; LB	PCR-RFLP	9
Aller R 16	2010	Spain	Caucasians	-308G/A (rs1800629)	66	203	HB	LB	PCR	8
Zhou YJ [23]	2010	China	Asian	-308 (rs1800629), -238(rs361525)	117	117	PB	US	PCR-RFLP	8
Trujillo-Murillo K [24]	2011	Mexico	Caucasians	-308 (rs1800629), -238(rs361525)	68	100	PB	LB	PCR-RFLP	6
Hegazy MA [20]	2016	Egypt	Caucasians	-238(rs361525)	100	30	HB	LB	PCR-RFLP	7
Mohseni F [17]	2016	Iran	Caucasians	-238(rs361525)	75	76	HB	US	Direct sequencing	7
Bocsan IC [21]	2017	Romania	Caucasians	-308 (rs1800629)	66	30	HB	LB	PCR-RFLP	7
Purnomo HD [18]	2018	Indonesia	Asian	-308(rs1800629), -238(rs361525)	75	75	HB	US	PCR-RFLP/direct sequencing	7
Damavandi N [26]	2021	Iran	Caucasians	-308 (rs1800629)	242	324	HB	US	ARMS-PCR	7
Bulatova IA [19]	2022	Rusia	Caucasians	-308(rs1800629)	52	65	HB	US	Real-time PCR	7

PB = Population-based, HB = Hospital-based, LB = Liver biopsy, US = Liver ultrasonographic, PCRRFLP = Polymerase chain reaction-restriction fragment length polymorphism, ARMS-PCR = Amplification refractory mutation system-polymerase chain reaction, NOS=Newcastle-Ottawa scale.

Table 2: Genotype distribution of different tumor necrosis factor- α (TNF-α) gene polymorphisms:

Included studies	Genotypes in cases (*n*)			Genotypes in controls (*n*)			HWE P-value
	G/G	G/A	A/A	G/G	G/A	A/A	
Tumor necrosis factor- α (TNF-α) gene polymorphism in the region -308 G/A (rs1800629)							
Valenti L 2002 [25]	75	23	1	132	38	2	0.69
Tokushige K 2007 [14]	97	4	1	95	5	0	0.80
Wong VW 2008 [15]	66	13	0	30	10	0	0.37
Hu ZW 2009 [22]	163	26	0	123	15	0	0.50
Aller R 2010 [16]	51	15	0	154	49	0	0.05
Zhou YJ 2010 [23]	106	11	0	110	7	0	0.74
Trujillo-MurilloK 2011 [24]	54	14	0	80	20	0	0.27
Bocsan IC 2017 [21]	52	11	3	23	6	1	0.46
Purnomo HD 2018 [18]	74	1	0	72	3	0	0.86
Damavandi N 2021 [26]	186	50	6	284	36	4	0.03
Bulatova IA 2022 [19]	7	41	4	14	51	0	<0.001
Tumor necrosis factor- α (TNF-α) gene polymorphism in the region -238 G/A (rs361525)							
	G/G	**G/A**	**A/A**	**G/G**	**G/A**	**A/A**	
Valenti L 2002 [25]	68	31	0	146	23	3	0.08
Tokushige K 2007 [14]	97	5	0	92	8	0	0.68
Wong VW 2008 [15]	74	5	0	36	4	0	0.74
Hu ZW 2009 [22]	137	52	0	116	22	0	0.31
Zhou YJ 2010 [23]	82	35	0	99	18	0	0.37
Trujillo-Murillo K 2011 [24]	48	20	0	88	12	0	0.52
Hegazy MA 2016 [20]	65	30	5	27	3	0	0.77
Mohseni F 2016 [17]	71	4	0	68	8	0	0.63
Purnomo HD 2018 [18]	72	3	0	73	2	0	0.91
Tumor necrosis factor- α (TNF-α) in the region -863 C/A (rs1800630)							
	C/C	**C/A**	**A/A**	**C/C**	**C/A**	**A/A**	
Tokushige K 2007 [14]	80	22	0	79	17	4	0.03
Wong VW 2008 [15]	51	28	0	27	13	0	0.22

HWE = Hardy Weinberg equilibrium

Meta-analysis of association between TNF-α -308G/A (rs1800629) polymorphism and NAFLD risk:

Eleven studies with 1155 NAFLD cases and 1364 controls demonstrated a significant association between TNF-α rs1800629 polymorphism and NAFLD under each of the dominant model (GA/AA vs. GG) [OR = 1.27, 95% CI = 1.01–1.59, P = 0.04], and allelic model (G vs. A) [OR = 1.26, 95% CI = 1.03–1.54, P = 0.02]. However, this significant association could not be observed under the heterozygous comparison (Table 3 and Figure 2A-C).

Table 3: Meta-analysis of associations of TNF-α -308G/A (rs1800629), -238G/A (rs361525), and -863C/A (rs1800630) polymorphisms with risk of nonalcoholic fatty liver disease.

Genetic model	OR [95% CI]	Z (*P* value)	Heterogeneity of study design			Meta-analysis model
			χ^2	df (*P* value)	I^2 (%)	
TNF-α -308G/A (rs1800629) polymorphism						
TNF-α -308G/A (rs1800629) polymorphism in total population from11 case control studies (1155cases and 1364controls) [14–16,18,19,21–26].						
Allelic model (G-allele vs. A-allele)	1.26 [1.03, 1.54]	2.30 (P=0.02)	11.38	10 (P = 0.33)	12%	Fixed
Dominant model (GA/AA vs. GG)	1.27 [1.01, 1.59]	2.07 (P=0.04)	12.06	10 (P = 0.28)	17%	Fixed
Heterozygous model (GA vs. GG)	1.24 [0.98, 1.56]	1.82 (P=0.07)	11.66	10 (P = 0.31)	14%	Fixed
TNF-α -308G/A (rs1800629) polymorphism in Caucasian population from 6 case control studies (593cases and 894controls) [16,19,21,24–26].						
Allelic model (G-allele vs. A-allele)	1.42 [1.12, 1.79]	2.88(P=0.004)	5.75	5(P = 0.33)	13%	Fixed
Dominant model (GA/AA vs. GG)	1.36 [1.05, 1.78]	2.30(P=0.02)	7.41	5 (P = 0.19)	33%	Fixed
Heterozygous model (GA vs. GG)	1.33 [1.02, 1.75]	2.09(P=0.04)	6.86	5 (P = 0.23)	27%	Fixed
TNF-α -308G/A (rs1800629) polymorphism in Asian population from 4 case control studies (562cases and 470controls) [14,15,18,22,23].						
Allelic model (G-allele vs. A-allele)	0.96 [0.66, 1.39]	0.23 (P=0.82)	2.65	4 (P = 0.62)	0%	Fixed
Dominant model (GA/AA vs. GG)	1.05 [0.68, 1.62]	0.22 (P=0.83)	3.66	4 (P = 0.45)	0%	Fixed
Heterozygous model (GA vs. GG)	1.03 [0.66, 1.58]	0.11 (P=0.91)	3.82	4 (P = 0.43)	0%	Fixed
TNF-α -308G/A (rs1800629) polymorphism in 9 case control studies that have achieved Hardy Weinberg Equilibrium among control groups (861cases and 975controls) [14–16,18,21–25].						
Allelic model (G-allele vs. A-allele)	1.02 [0.79, 1.32]	0.18(P=0.86)	3.61	8(P = 0.89)	0%	Fixed
Dominant model (GA/AA vs. GG)	1.01 [0.77, 1.33]	0.10(P=0.92)	3.85	8 (P = 0.87)	0%	Fixed
Heterozygous model (GA vs. GG)	1.00 [0.76, 1.32]	0.01(P=0.99)	4.08	8(P=0.85)	0%	Fixed
TNF-α -308G/A (rs1800629) polymorphism in 2 case control studies that couldn't achieve Hardy Weinberg Equilibrium among control groups (294cases and 389controls) [19,26].						
Allelic model (G-allele vs. A-allele)	1.75 [1.27, 2.41]	3.42 (P=0.0006)	1.27	1 (P = 0.26)	21%	Fixed
Dominant model (GA/AA vs. GG)	2.07 [1.38, 3.11]	3.50 (P=0.0005)	0.12	1 (P = 0.73)	0%	Fixed
Heterozygous model (GA vs. GG)	2.01 [1.32, 3.07]	3.24 (P=0.001)	0. 24	1 (P = 0.62)	0%	Fixed
TNF-α -238 G/A (rs361525) polymorphism						
TNF-α -238 G/A (rs361525) polymorphism in total population from9 case control studies (904cases and 848controls) [14,15,17,18,20,22–25].						
Allelic model (G-allele vs. A-allele)	1.66 [1.13, 2.44]	2.56 (P=0.01)	14.40	8 (P = 0.07)	44%	Random
Dominant model (GA/AA vs. GG)	1.76 [1.14, 2.71]	2.57 (P=0.01)	16.17	8 (P = 0.04)	51%	Random
Heterozygous model (GA vs. GG)	1.77 [1.14, 2.74]	2.57 (P=0.01)	16.31	8 (P = 0.04)	51%	Random
TNF-α -238 G/A (rs361525) polymorphism in Caucasian population from 4 case control studies (342cases and						

378controls) [17,20,24,25]**.**						
Allelic model (G-allele vs. A-allele)	1.98 [0.99, 3.97]	1.93(P=0.05)	7.61	3(P = 0.05)	61%	Random
Dominant model (GA/AA vs. GG)	2.20 [1.03, 4.66]	2.05(P=0.04)	8.02	3 (P = 0.05)	63%	Random
Heterozygous model (GA vs. GG)	2.22 [1.05, 4.70]	2.08(P=0.04)	7.85	3 (P = 0.05)	62%	Random
TNF-α -238 G/A (rs361525) polymorphism in Asian population from 5 case control studies (562cases and 470controls) [14,15,18,22,23]**.**						
Allelic model (G-allele vs. A-allele)	1.60 [1.13, 2.26]	2.65(P=0.008)	5.86	4 (P = 0.21)	32%	Fixed
Dominant model (GA/AA vs. GG)	1.69 [1.17, 2.43]	2.81(P=0.005)	6.68	4 (P = 0.15)	40%	Fixed
Heterozygous model (GA vs. GG)	1.69 [1.17, 2.43]	2.81(p=0.005)	6.68	4(P=0.15)	40%	Fixed
TNF-α -863 C/A (rs1800630) polymorphism						
TNF-α -863 C/A (rs1800630) polymorphism in total population from 2 case control studies (181cases and 140 controls) [14,15]**.**						
Allelic model (G-allele vs. A-allele)	0.95 [0.60, 1.51]	0.23 (P=0.82)	0.32	1 (P = 0.57)	0%	Fixed
Dominant model (GA/AA vs. GG)	1.08 [0.64, 1.81]	0.28 (P=0.78)	0.08	1(P = 0.86)	0%	Fixed
Heterozygous model (GA vs. GG)	1.22 [0.72, 2.07]	0.72 (P=0.47)	0.04	1 (P = 0.83)	0%	Fixed

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval.

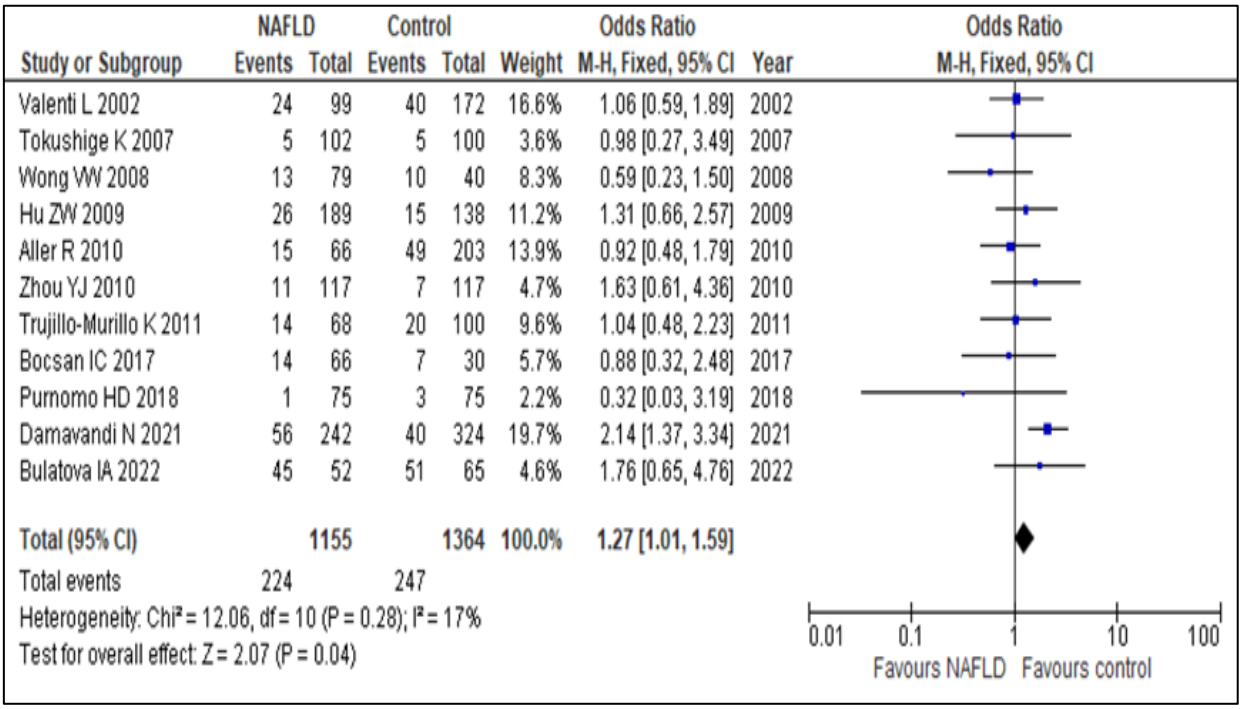

Fig. 2.A: Forest plot for the association between NAFLD risk association and TNF-α gene polymorphism in region -308G/A (rs1800629) under the dominant model in the total population.

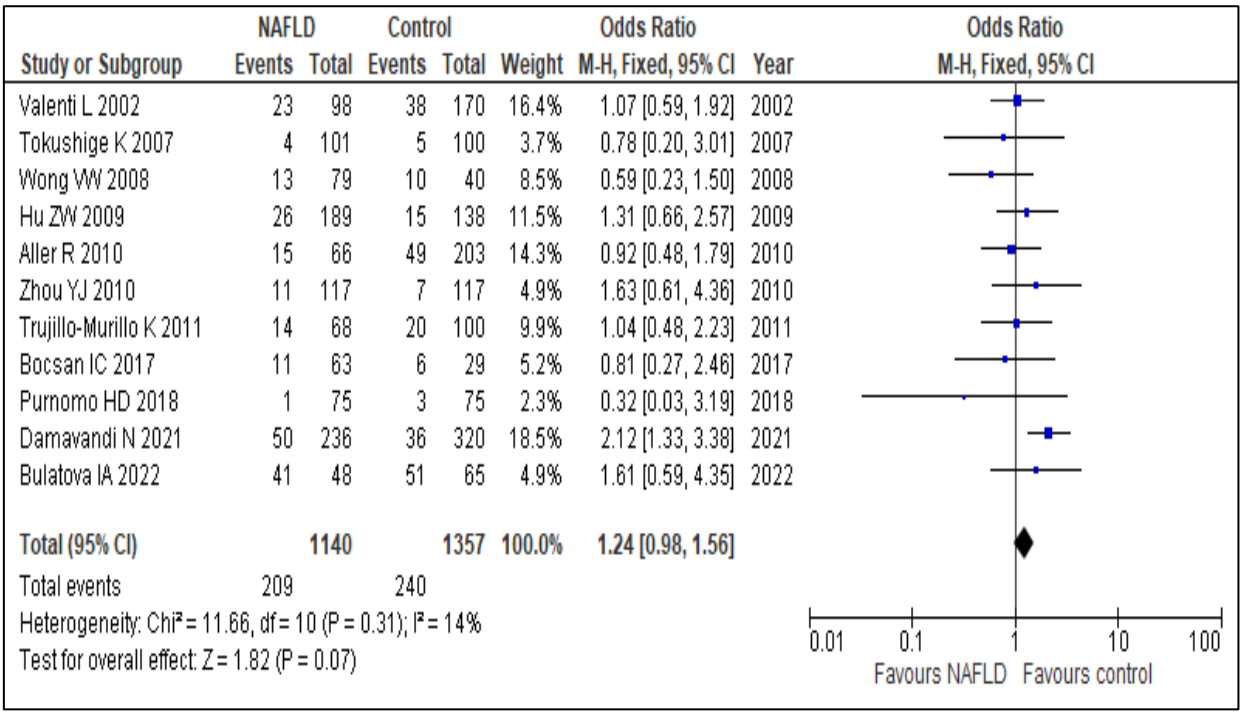

Fig. 2.B: Forest plot for the association between NAFLD risk association and TNF-α gene polymorphism in region -308G/A (rs1800629) under the heterozygous model in the total population.

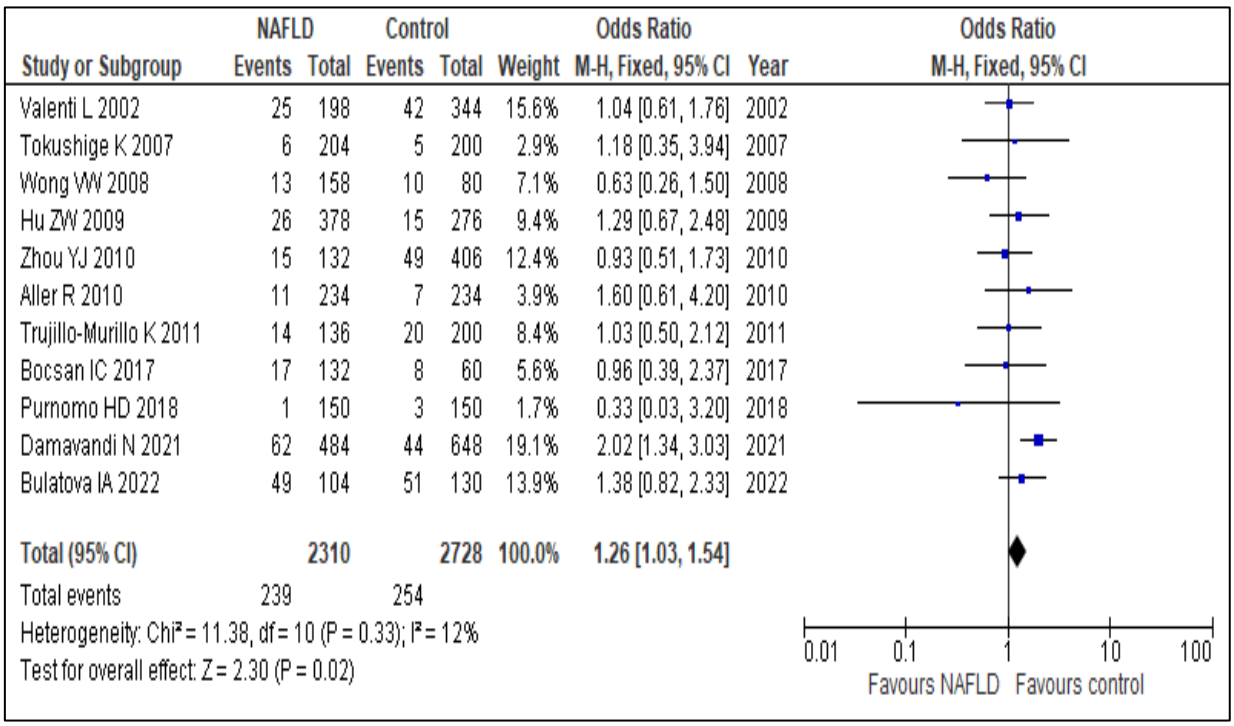

Fig. 2.C: Forest plot for the association between NAFLD risk association and TNF-α gene polymorphism in region -308G/A (rs1800629) under the allelic model in the total population.

Subgroup analysis:

1) Subgroup analysis based on ethnicity:

After stratifying the included studies based on ethnicity, 6 studies conducted on Caucasians with 593 NAFLD cases and 894 controls were included in the final analysis. The analysis among Caucasians, demonstrated the significant association between TNF-α rs1800629 polymorphism and NAFLD under all tested models; dominant model with [(OR= 1.36, 95%CI=1.05-1.78), P=0.02)]; heterozygous model with [(OR= 1.33, 95%CI=1.02-1.75), P=0.04)]; the Allelic model with [(OR= 1.42, 95%CI=1.12-1.79), P=0.004)]. However, this significant relation could not be concluded within the studied Asian population (Table 3).

2) Subgroup analysis based on HWE:

Meta-analysis of the nine studies achieved HWE demonstrated no significant association between the rs1800629 SNP and NAFLD under all tested models.

In contrast, the analysis including the 2 studies didn't achieve HWE, suggested a significant association through all tested models; the dominant model with [(OR= 2.07, 95%CI=1.38-3.11, P value=0.0005)]; the heterozygous model with [(OR= 2.01, 95%CI=1.32-3.07, P value=0.001)]; the allelic model [(OR= 1.75, 95%CI=1.27-2.41, P value=0.0006)] (Table 3).

Meta-analysis of association between TNF-α -238G/A (rs361525) polymorphism and NAFLD risk:

Nine studies with 904 NAFLD cases and 848 controls demonstrated the significant association between TNF-α gene rs361525 polymorphism and NAFLD with significant heterogeneity across studies. This relation was consistent under each of the dominant model (GA/AA vs. GG) [OR = 1.76, 95% CI = 1.14–2.71, P =0.01], heterozygous model GA vs. GG [OR = 1.77, 95% CI = 1.14–2.74, P = 0.01], and allelic model (G vs. A) [OR = 1.66, 95% CI = 1.31–2.44, P = 0.01] (Table 3 and Figure 3A-C).

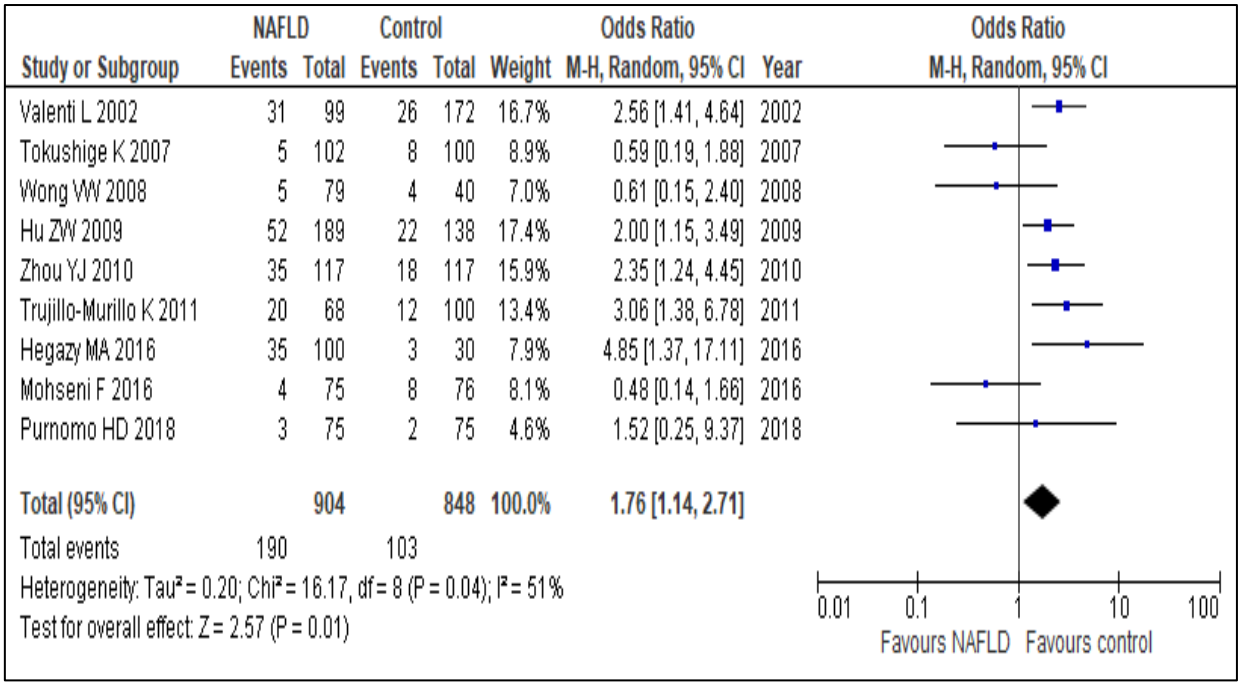

Fig. 3.A: Forest plot for the association between NAFLD risk association and TNF-α gene polymorphism in region -238 G/A (rs361525) under the dominant model in the total population.

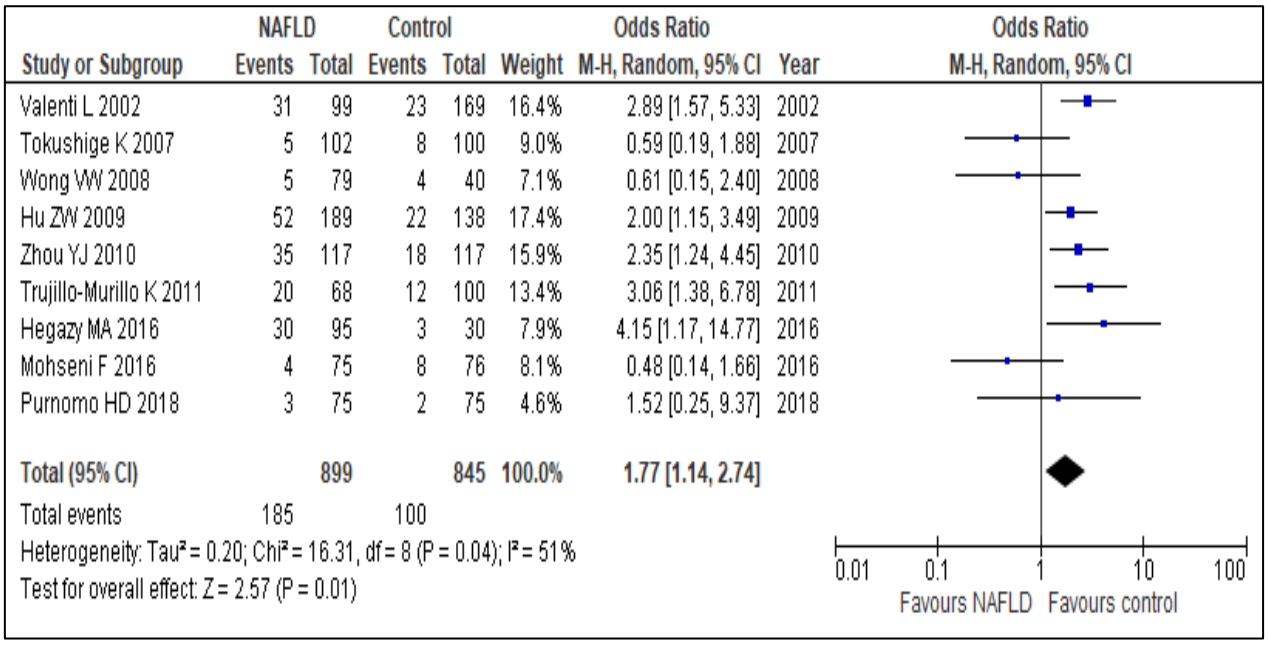

Fig. 3.B: Forest plot for the association between NAFLD risk association and TNF-α gene polymorphism in region -238 G/A (rs361525) under the heterozygous model in the total population.

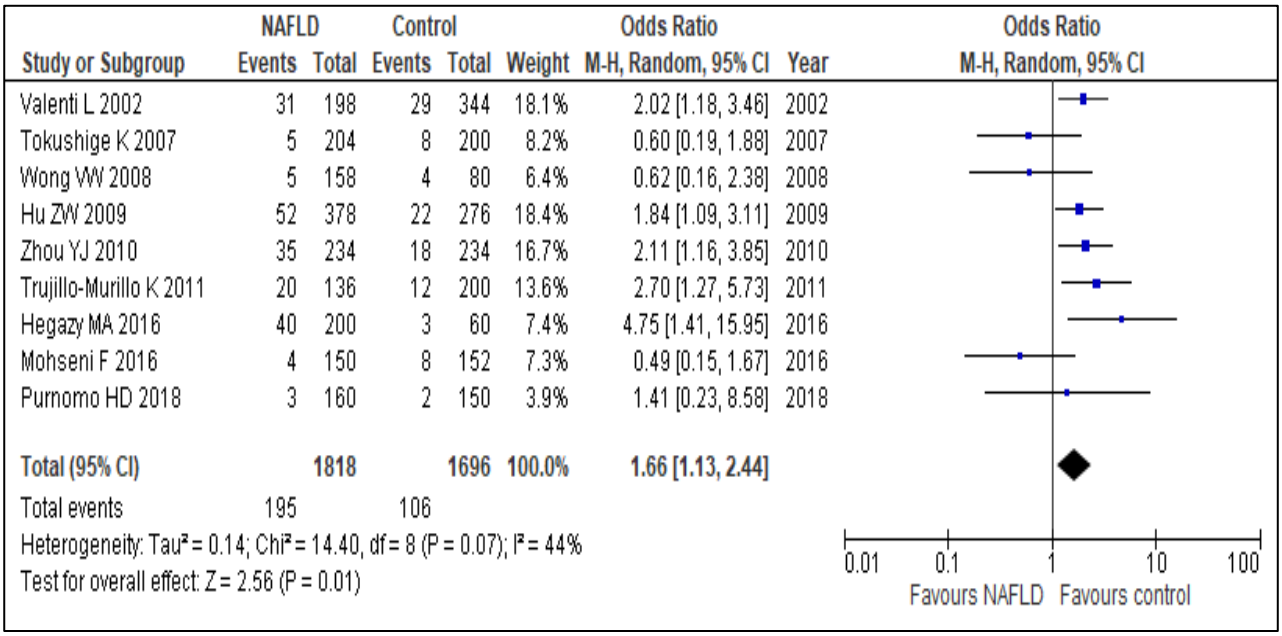

Fig. 3.C: Forest plot for the association between NAFLD risk association and TNF-α gene polymorphism in region -238 G/A (rs361525) under the allelic model in the total population.

Subgroup analysis:

After stratifying the included studies based on ethnicity, 4 studies conducted on Caucasians with 342 NAFLD cases and 378 controls were included in the final analysis. The analysis among Caucasians, demonstrated the significant association between rs361525 polymorphism and NAFLD using the random model under each of; dominant model with [(OR= 2.20, 95%CI=1.03, 4.66), P=0.04)]; and heterozygous model with [(OR= 2.22, 95%CI=1.05, 4.70), P=0.04)]. Nevertheless, the Allelic model showed that this association was insignificant (Table 3).

Lastly, the remaining 5 studies included Asian population were meta analyzed together with 562 NAFLD cases and 470 controls. The analysis among Asians, demonstrated the significant association

between TNF-α gene rs361525 polymorphism and NAFLD under all tested models using the fixed model; dominant model with [(OR= 1.69, 95%CI=1.17, 2.43), P=0.005)]; heterozygous model with [(OR= 1.69, 95%CI=1.17, 2.43), P=0.005)]; the Allelic model with [(OR= 1.60, 95%CI=1.13, 2.26), P=0.008)] (Table 3).

Meta-analysis of association between TNF-α gene -863 C/A (rs1800630) polymorphism and NAFLD risk:

The Meta-analysis included two studies with total of 181 NAFLD cases and 140 controls suggested that there was no enough evidence of association between TNF-α gene rs1800630 polymorphism and NAFLD risk under each of the examined inheritance models (Table 3 and Supplementary Figure S1).

Publication bias: Publication bias was assessed for rs1800629 SNP using both funnel plot and Egger's test (Supplementary Table S1 and Supplementary Figure S2). Review of the symmetry of funnel plots could not get rid of the potential for publication bias for analysis. Therefore, the publication bias was further evaluated by Egger's test. Egger's test suggested that there was no risk of publication bias in the dominant model, while each of heterozygous and the allelic models showed potential risk of publication bias.

Sensitivity Analysis:

To assess the reliability of the outcomes in the meta-analysis, we repeated the meta-analysis after excluding, one by one, two studies (that didn't achieve Hardy-Weinberg equilibrium in which the P value associated with HWE was less than 0.05 [19, 26].

After excluding the study by Bulatova et al. [19], the results did not differ substantially in total population except for the allelic model, while in Caucasian population for rs1800629 polymorphism results were altered in the heterozygous model (Supplementary Table S2).

However, after excluding the study by Damavandi & Zeinali [26], the results changed in all models in total and Caucasian population for rs1800629 polymorphism (Supplementary Table S3).

DISCUSSION

NAFLD is one of the most common hepatic diseases worldwide. Pathogenesis of NAFLD is multifactorial and has a significant genetic background. Polymorphism of the TNF alpha gene is a strong candidate risk factor for the disease that may affect the TNF alpha expression, that is considered an important cytokine in the pathogenesis of NAFLD and known as a regulator for IR.

Genetic association studies examined the association of TNF alpha gene polymorphisms and increased NAFLD risk have reported contradictory results. The current study aimed to summarize the literature to assess the association between different TNF alpha candidate gene polymorphisms and susceptibility to NAFLD.

The previous systematic review that studied the relationship between TNF alpha gene polymorphisms and NAFLD included eight studies. Wang and his colleagues concentrated their search on the two TNF alpha promoter gene polymorphisms at positions -308 and -238, with meta-analysis examining only the dominant inheritance model [29]. Several relevant studies have been published after the previous systematic review [18–20, 26]. Therefore, this study is an important update to the scientific literature.

The current systematic review searched for all relevant studies that investigated the association of any candidate TNF alpha polymorphisms with susceptibility to NAFLD. In addition, we include all studies involved in the previous systematic review except for one study published in the Chinese language due to the language barrier [43].

The current meta-analysis tested various inheritance models: dominant, heterozygous, and allelic models to examine the association of included polymorphisms with NAFLD risk.

The systematic search revealed that throughout all TNF-α gene polymorphisms, five SNPs have been studied in relation to NAFLD risk: TNF-α -1031 (rs1799964), -863(rs1800630), -857(rs1799724), -308(rs1800629), and -238 (rs361525).

Considering that the minimum number of studies to conduct a meta-analysis is two, there were only three TNF- α SNPs at positions -308(rs1800629), -238 (rs361525), & -863 (rs1800630) eligible for meta-analyses with a total of 13 studies [14–26].

Using the NOS, the quality of included studies scored ≥6 stars or more, indicating good quality. Examination of HWE of genotype distributions among controls in the included studies is another important aspect for assessing the quality of the genetic association studies. All included studies achieved HWE, except two studies included in rs1800629 analyses [19, 26], with one study involved in rs1800630 meta-analyses failed to achieve HWE [14].

This study provides additional evidence about the association between TNF alpha polymorphisms and the susceptibility to NAFLD. Results demonstrated that there is a significant association between rs1800629 polymorphism and increased risk for NAFLD among all studied populations under all tested genetic models except for the heterozygous model. Stratifying the studied population based on ethnicity revealed that this relation remained significant among the Caucasian population across all the studied genetic models. On the other hand, none of these studied models suggested this significant relation among the Asian population.

None of the included studies suggested a significant association between the rs1800629 SNP and the

increased risk for NAFLD except for the study conducted by Damavandi & Zeinali [26].

The exclusion of the Damavandi & Zeinali s' study[26] altered the results significantly. That may be attributed to its relatively large sample size compared to other included studies. Besides, it's the only study (among all included studies) that suggested a significant association between the rs1800629 and the increased susceptibility to NAFLD. Sensitivity analysis revealed that the results of the rs1800629 meta-analyses should be generalized to the broader population cautiously. Therefore, the results of the meta-analysis including only the studies that achieved HWE more reliable suggesting that there is no enough evidence of the association between the -308(rs1800629) SNP and susceptibility to NAFLD in accordance with the previous meta-analysis. This discrepancy in results highlights the importance of conducting more studies with sufficient sample size and proper selection of included subjects to further study this association.

The current meta-analysis demonstrated that rs361525 polymorphism was significantly associated with the risk of NAFLD under all tested inheritance models with heterogeneity across studies. This significant relation persisted across different ethnic groups examined except for the allelic comparison among the Caucasian population.

The current analysis results are consistent with the previous meta-analysis suggesting a significant association between the rs361525 SNP and the susceptibility for NAFLD.

Meanwhile, several included studies have concluded similar results [20,22,24,25], other included studies couldn't find a significant difference between NAFLD cases and controls regarding the rs361525 SNP [14,15,17,18].

After stratification based on race the degree of heterogeneity became insignificant among the Asian population while remained significant within the Caucasian population, suggesting that the diversity across Caucasian studies may be the main source of heterogeneity in the meta-analysis of total population.

Meta-analysis of only two studies concluded that there is no enough evidence of association between rs1800630 SNP and increased risk to NAFLD.

There were several limitations to this meta-analysis. First, the limited number of relevant studies resulted in a relatively small sample size for the current study. This relatively small number of included subjects might have affected the statistical power of this study to detect the association between TNF-α SNPs and susceptibility to NAFLD. In addition, three of the included studies couldn't achieve HWE. Accordingly, the results of the meta-analyses of -308 (rs1800629) and -863 (rs1800630) SNPs should be interpreted with caution. Moreover, the discrepancy of sensitivity and specificity of the different methods used for NAFLD diagnosis and genotyping across studies may affect the robustness of this meta-analysis. Lastly, limited published data about other risk factors; obesity, family history of type 2 diabetes, insulin resistance, and metabolic syndrome in original articles, may affect our final conclusions.

Declarations:

Consent for publication: Not applicable

Availability of data and material: Data are available upon request.

Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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CONCLUSION

This meta-analysis suggested that TNF-α gene polymorphisms rs361525 and rs1800629, but not rs1800630, might be a risk factor for NAFLD. That may explain the reported increase in the circulating TNF alpha levels among NAFLD patients compared to healthy population. These results give new insights for NAFLD diagnosis and treatment. This study highlighted that the possible association between TNF alpha gene polymorphisms and NAFLD risk needs further investigation with larger sample size and more appropriate subjects' selection.

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