Systematic Review and Meta-Analysis

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Associations between methylenetetrahydrofolate reductase polymorphisms and hepatocellular carcinoma risk

An update meta-analysis and trial sequential analysis

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Abstract

Aim: To evaluate the associations between the methylenetetrahydrofolate reductase (MTHFR) single-nucleotide polymorphisms (SNPs) and hepatocellular carcinoma (HCC) with meta-analysis and trial sequential analysis.

Methods: PubMed, Embase, the Google Scholar, Wan fang database, VIP database, and China National Knowledge Infrastructure were extensively searched before April 2021. Odds ratios (ORs) and 95% confidence interval (95% CI) were calculated. Review Manager Version 5.3, STATA version 12.0 and TSA 0.9.5.10 Beta software were used.

Results: Nineteen studies with 6941 HCC patients and 9436 controls were finally included. The MTHFR rs1801133 (C677T) SNP was associated with increased HCC risk under heterozygote genetic model (OR = 1.10, 95% CI = [1.01, 1.20]). For Subgroup analysis, increased risks of HCC were detected in Mongoloid, Chinese. For MTHFR rs1801131 (A1298C) SNP, increased risk of HCC was only observed in Caucasians (allelic: OR = 1.86, 95% CI = [1.49, 2.31]; homozygote: OR = 3.39, 95% CI = [2.18, 5.27]), interesting decreased risk was detected in Mongoloid (recessive: OR = 0.30, 95% CI = [0.15, 0.58]; homozygote: OR = 0.41, 95% CI = [0.24, 0.72]). Sensitivity analysis indicated stability in our results. Publication bias was not detected based on Begg test and Egger test. Trial sequential analysis indicated further studies to confirm the associations in MTHFR C677T polymorphism.

Conclusion: The MTHFR rs1801133 SNP was associated with an increased risk of HCC in Mongoloid population especially in Chinese. Increased HCC risk is also observed in Caucasian population for the MTHFR rs1801131 SNP, and decreased risk of HCC is remarkably discovered in Mongoloid and Chinese subgroups, which need further validation.

Abbreviations: CI = confidence interval, HCC = hepatocellular carcinoma, MTHFR = methylenetetrahydrofolate reductase, ORs = odds ratios, SNP = single-nucleotide polymorphism.

Keywords: hepatocellular carcinoma, meta-analysis, methylenetetrahydrofolate reductase, polymorphism, trial sequential analysis

1. Introduction

Hepatocellular carcinoma (HCC) accounts for roughly 90% of all primary liver cancers, which is the sixth most common cancer and the second leading cause of cancer death worldwide.[1–5] HCC is asymptomatic most of the time, and when symptoms appear patients are usually at the middle or late stage, which result in a high mortality.[6–9] Therefore, early diagnosis based on related risk factors is of great significant to prevent HCC. HCC is a multifactorial disease due to the complex interactions between genetic and environmental factors. Genetic polymorphisms in HCC related genes such as toll-like receptor genes,[10] PD-L1,[11] matrix metalloproteinase-11 gene,[12] have drawn increasing attention in the past decades. To filtrate predisposing gene polymorphisms is important to the early prevention of HCC.

Folate metabolism plays an important role in the DNA synthesis and methylation, which is crucial to the development of HCC. Methylenetetrahydrofolate reductase (MTHFR) is the key enzyme in folate and one-carbon metabolism, which can catalyze the 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The 5-methyltetrahydrofolate is the predominant circulatory form of folate and serves as the methyl donor for the re-methylation of homocysteine to methionine, which is the precursor of S-adenosylmethionine (SAMe), the principal biological methyl donor for methylation of DNA.[13–18] Two functional single nucleotide polymorphisms (SNPs) in MTHFR were identified: the MTHFR rs1801133 (C677T) polymorphism (a C to T transition at nucleotide 677 at exon 4, resulting in an alanine-to-valine conversion in protein) and MTFHR rs1801131 (A1298C) polymorphism (a A to C transition at nucleotide 1298 at exon ten, causing a glutamate-to-alanine change in

Editor: Yan Li.

The authors have no conflicts of interests to disclose.

Supplemental Digital Content is available for this article.

The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

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How to cite this article: Wang B, Ma M, Guo X, Yan Y, Li L. Associations between methylenetetrahydrofolate reductase polymorphisms and hepatocellular carcinoma risk: an update meta-analysis and trial sequential analysis. Medicine 2021;100:41(e27527).

Received: 8 October 2018 / Received in final form: 28 September 2021 / Accepted: 28 September 2021

http://dx.doi.org/10.1097/MD.0000000000027527

protein).[19,20] Both of the 2 polymorphisms were reported to be associated with a lower MTHFR activity,[21–28] and the reduced enzymatic activity can promote or inhibit the occurrence of HCC by affecting DNA methylation and synthesis, which indicated these 2 polymorphisms could be associated with HCC risk.

Many studies were performed to discover the associations between the 2 MTHFR SNPs and HCC risk, however, the conclusions were inconclusive.[13,29,30] Former meta-analyzes in 2014 and 2015 reported the MTHFR rs1801133 polymorphism was associated with an increased risk of HCC, but for the MTHFR rs1801131 polymorphism, no association was observed.[30,31] Since then, controversial results emerged in different regions and ethnicities.[13,29,32–34] In order to reach a more accurate evaluation of these 2 polymorphisms and HCC risk, we performed an update meta-analysis with trial sequential analysis.

2. Methods

Based on the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) checklist,[35] we organized our update meta-analysis. Ethical approval was not necessary for the type of the study (meta-analysis).[36]

2.1. Literature search

An extensive literature search for related studies regarding the associations between MTHFR polymorphisms and hepatocellular carcinoma risk was conducted on PubMed, Embase, the Google Scholar, Wan fang database, VIP database, and China National Knowledge Infrastructure before April 2021. We used the following keywords and MeSH terms: "Methylenetetrahydrofolate reductase" or "folate metabolism" or "one-carbon metabolism" or "MTHFR," "polymorphism, single nucleotide" or "single nucleotide polymorphism" or "polymorphism" or "SNP," and "carcinoma, hepatocellular" or "liver neoplasms" or "liver cancer." No language restrictions were set in our search, furthermore, references of eligible studies were screened manually to identify potential relevant researches.

2.2. Inclusion and exclusion criteria

Studies included should meet the following inclusion criteria:

1. case-control studies or cohort studies;
2. studies on analyzing the associations between MTHFR polymorphisms and hepatocellular carcinoma risk;
3. hepatocellular carcinoma patients should be diagnosed by histopathology in the included study;
4. studies providing detailed genotype frequencies on case and control subjects.

The exclusion criteria were as follows:

1. reviews, comments and conference documents;
2. unclear diagnostic basis for case subjects;
3. animal research;
4. studies with insufficient data, especially without enough data for Hardy-Weinberg equilibrium;
5. duplicate publications.

2.3. Data extraction and quality assessment

Data from the potential eligible studies were independently retrieved by all the authors based on a pre-designed standard form. The following data were extracted: name of the first author, year of publication, country and region where the study was conducted, matching criteria, ethnicity, genotyping method, source of controls, genotype frequency in the cases and controls, quality score and results of Hardy-Weinberg equilibrium test. Ethnicity was categorized as Mongoloid, Caucasian. Hardy–Weinberg equilibrium (HWE) was evaluated for each study by Chi-Squared test in control groups for goodness of fit, and $P < .05$ was considered as a significant departure from HWE. Any disagreement was resolved by group discussion. The quality assessment for each eligible study was assessed based on the modified Newcastle-Ottawa quality assessment scale.[37] Scores ranged from 0 to 10, with 0 as the lowest and 10 as the highest quality.

2.4. Statistics analysis

Odds ratio (OR) and 95% confidence intervals (CIs) were calculated to evaluate the strength of the association between MTHFR gene polymorphisms and hepatocellular carcinoma risk. Pooled ORs were performed for allelic model (rs1801133: T VS C; rs1801131: C VS A), recessive model (rs1801133: TT VS TC+CC; rs1801131: CC VS CA+AA), dominant model (rs1801133: TT+TC VS CC; rs1801131: CC VS CA+AA), heterozygote model (rs1801133: TC VS CC; rs1801131: CA VS AA), homozygote model (rs1801133: TT VS CC; rs1801131: CC VS AA), respectively. Heterogeneity was evaluated by Q statistic (significance level of $P < .1$) and I^2 statistic (greater than 50% as evidence of significant inconsistency). If the P value of heterogeneity test was more than .1 or I^2 statistic less than 50%, the fixed-effect model was used to calculate the pooled OR, otherwise, the random-effect model was used. Sensitivity analysis was performed to detect the heterogeneity by omitting each study in each turn. In addition, subgroup analyses were stratified by HWE (Whether meet the Hardy Weinberg Equilibrium), Region (China, France, Italy, South Korea, USA, Brazil), ethnicity (Mongoloid, Caucasian), source of controls (Hospital based, Population based). The potential for publication bias was assessed with Begg funnel plot and Egg test. All the tests in this meta-analysis were conducted with Review Manager Version 5.3 and the STATA software (version 12.0; State Corporation, College Station, Texas, USA). All tests were two-sided and a P value of lower than .05 was considered as statistically significant.[38]

2.5. Trial sequential analysis

Due to dispersed data and repeated significance testing, type I and type II errors are inevitable in traditional updated meta-analysis with new trials.[39] Bias from trials with low methodological quality, outcome measure bias, early stopping for benefit, and small trial bias may result in spurious P values, therefore, the trial sequential analysis which is a methodology that combines an information size calculation (cumulated sample sizes of all included studies) for an updated meta-analysis with the threshold of statistical significance was introduced in our analysis (http://www.ctu.dk/tools-and-links/trial-sequential-analysis.aspx). The trial sequential analysis software (TSA, version 0.9; Copenhagen Trial Unit, Copenhagen, Denmark, 2011) was used and the parameters were set as an overall type I error of 5%, a statistical test power of 80%, and a relative risk reduction of 20%.[40–42] If the Z-curve crosses the trial sequential monitoring boundary line

or required information size is reached, a sufficient level of evidence has been acquired and no more further studies are needed, or else, further studies are essential.

3. Results

3.1. The characteristics of the included studies

A total of nineteen articles were included and 6941 hepatocellular carcinoma patients and 9436 healthy controls were enrolled[13,29,32–34,43–56] (The flow chat of literature selection was showed in Figure 1 and Table S1 (see Table S1, Supplemental Digital Content, which showed the flow chat of literature selection, http://links.lww.com/MD/G441). The characteristics of the included studies were showed in Table 1. Among the nineteen included articles, 20 studies were research regarding the associations between the MTHFR rs1801133 polymorphism (There were 2 different study groups from the article Yuan et al[49]) and hepatocellular carcinoma, and 8 studies were about the MTHFR rs1801131 polymorphism. For the MTHFR rs1801133 polymorphism, 5227 HCC patients and 6688 healthy controls were involved, which came from China, France, Italy, South Korea, USA and Brazil; as for the MTHFR rs1801131 polymorphism, 1714 HCC and 2775 healthy controls from

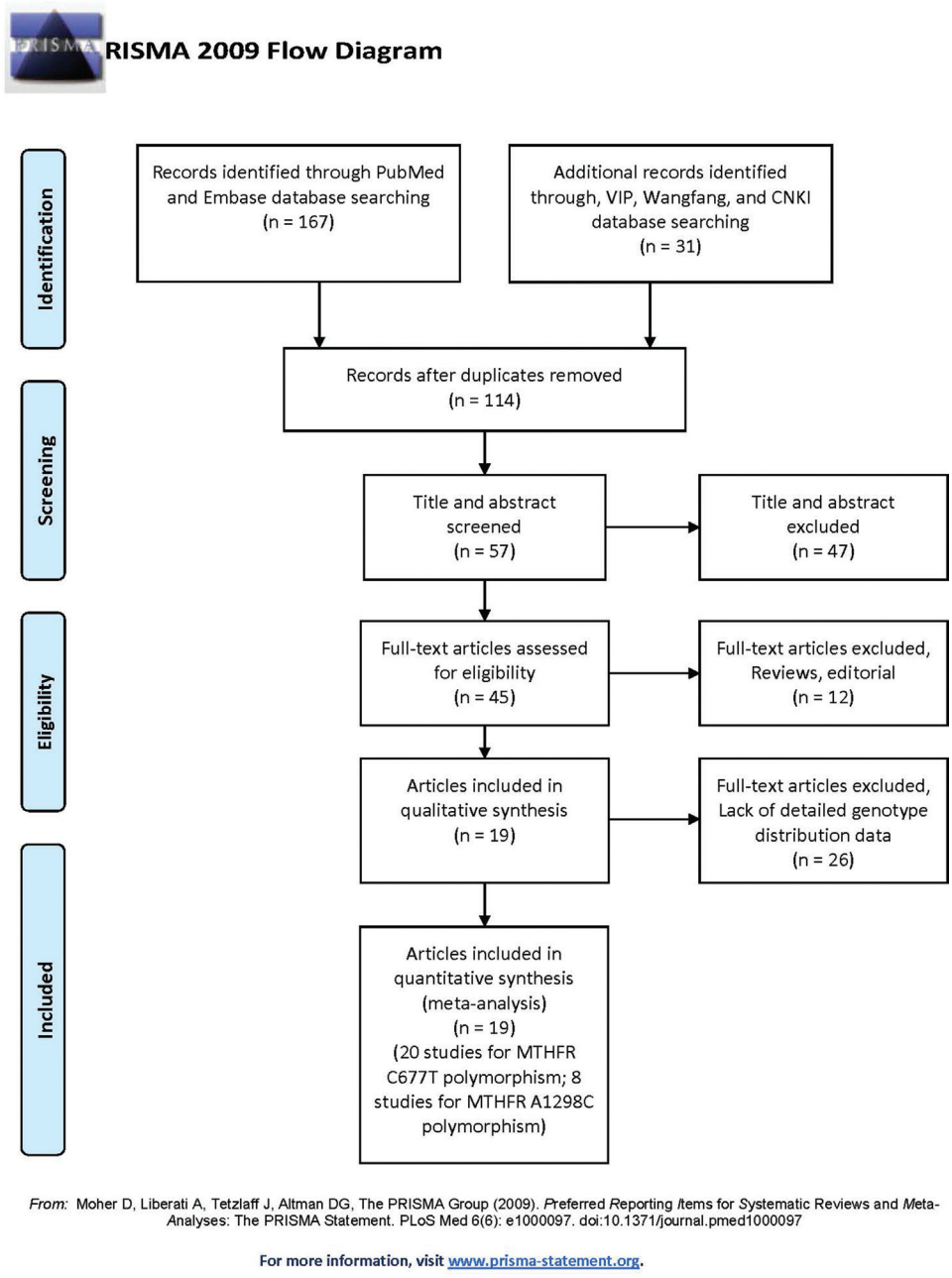

Figure 1. The flow chat of literature selection of the meta-analysis.

Table 1

Characteristics of included studies regarding the associations between MTHFR polymorphisms and HCC.

Study	Year	Country region	Matching criteria	Ethnicity	Genotyping method	Controls source	Sample size	HCC			Controls			Quality score	HWE*
								AA	AB	BB	AA	AB	BB		
***MTHFR* C677T polymorphism**															
Wang8	2018	China-Hangzhou	Geder, Age	Mongoloid	TaqMan	HB	731	80	121	43	164	216	107	8	0.026
Qiao32	2017	China-Tianjin	Gender, Age, BMI, Duration of HBV infection, HBV DNA, HCC family history	Mongoloid	PCR-DNA microarray-based assay	HB	415	30	100	74	40	120	51	7	0.041
Jiao33	2017	China-Tianjin	Gender, Age, HBsAg, HBV DNA, Child-Pugh stage, AFP, GGT-II, Drinking habit, Smoking habit	Mongoloid	TaqMan	HB	1275	168	370	188	110	263	176	9	0.514
Peres29	2016	Brazil-Sao Paulo	Age, Gender, Alcoholic habit, Sm oking habit	Caucasian	PCR-RFLP	HB	427	28	36	7	149	174	33	8	0.077
Lei54	2016	China-Enshi	Gender, Age, Smoking habit, HCC family history, HBV infection, Drinking habit, BMI	Mongoloid	PCR-RFLP	HB	250	19	38	64	12	32	85	6	0.002
Zhang30	2015	China-Shenyang	Gender, Age, HBV infection, HCV infection	Mongoloid	PCR-Illumina Golden Gate platform	HB	3000	440	800	260	498	770	232	8	0.020
Chang43	2014	China-Taixin	Gender, Age, BMI, Education, Smoking, Alcohol drinking, H. pylori ChagheA status, HBsAg status, Anti-HCV status, Plasma AFB1-albumin adduct levels	Mongoloid	PCR-RFLP	PB	585	50	114	30	135	199	57	10	0.235
Xu55	2014	China-Shanghai	Gender, Age, HBV infection	Mongoloid	PCR-SNaPshot	HB	405	50	112	43	50	111	39	8	0.109
Cui44	2012	China-Qingdao	Gender, Age, Drinking status, HBsAg	Mongoloid	PCR-SSCP	PB	997	52	179	125	121	325	195	10	0.483
Couvert45	2012	France-Paris	Gender, Age, Alcohol, BMI, Diabetes, Platelets count, Prothrombin time, Albumin, Bilirubin, ALT, HCV RNA load, HCV genotype	Caucasian	PCR-DHPLC	HB	121	23	29	10	26	23	10	8	0.223
Liu56	2010	China-Tianjin	Age, HBV infection status AFP, Duration of HBV infection	Mongoloid	TaqMan	HB	333	39	85	57	37	64	51	8	0.063
D'Amico47	2009	Italy-Palermo	Age, Duration of HBV infection	Caucasian	PCR-RFLP	PB	188	30	37	27	56	28	10	7	0.036
Fabris46	2009	Italy-Udine	Gender, Age at operation, Etiology, Child-Pugh Score, Presence of diabetes mellitus	Caucasian	TaqMan	HB	301	22	30	13	69	113	54	9	0.554
Kwak48	2008	South Korea-Seongnam	Age	Mongoloid	TaqMan	HB	297	32	46	18	64	106	31	7	0.234
YuanI49	2007	USA-Los Angeles	Gender, Age, Race/ethnicity, Level of education	Caucasian	TaqMan	HB	327	53	51	14	80	99	30	8	0.944
YuanII49	2007	China-Guangxi	Gender, Age, Race/ethnicity, Level of education	Mongoloid	TaqMan	HB	327	65	44	9	104	85	20	8	0.666
Mu50	2007	China-Taixin	Gender, Age, Education, Income	Mongoloid	PCR-RFLP	HB	585	50	114	30	135	199	57	9	0.235
Zhu51	2006	China-Shanghai	Gender, smoking habit, Drinking habit, HBsAg, Anti-HCV, HCC family history	Mongoloid	TaqMan	HB	1051	172	226	110	173	268	102	9	0.921
Ventura52	2005	Italy-Figgia	Age, BMI, Albumin, Quick, Creatinine, Haemoglobin, Platelet count	Caucasian	TaqMan	HB	72	7.986	4.994	9.02	27	16	7	8	0.092
Saffroy53	2004	France-Paris	Gender, Age	Caucasian	PCR-RFLP	HB	228	67	69	12	30	37	13	8	0.780
***MTHFR* A1298C polymorphism**															
Wang8	2018	China-Hangzhou	Gender, Age	Mongoloid	TaqMan	HB	731	181	57	6	296	140	51	7	0.000
Peres29	2016	Brazil-Sao Paulo	Age, Gender, Alcoholic habit, Smoking habit	Caucasian	PCR-RFLP	HB	427	32	24	15	205	116	35	7	0.003
Xu55	2014	China-Shanghai	Gender, Age, HBV infection	Mongoloid	PCR-SNaPshot	HB	405	150	52	3	152	44	4	8	0.698
Cui44	2012	China-Qingdao	Gender, Age, Drinking status, HBsAg	Mongoloid	PCR-SSCP	PB	997	258	94	4	461	153	27	9	0.003
Kwak48	2008	South Korea-Seongnam	Age	Mongoloid	TaqMan	HB	297	67	28	1	155	41	5	7	0.261
YuanI49	2007	USA-Los Angeles	Gender, Age, Race/ethnicity, Level of education	Caucasian	TaqMan	HB	549	159	71	71	156	74	18	8	0.033
YuanII49	2007	China-Guangxi	Gender, Age, Race/ethnicity, Level of education	Mongoloid	TaqMan	HB	495	136	101	10	136	91	21	8	0.305
Mu50	2007	China-Taixin	Gender, Age, Education, Income	Mongoloid	PCR-RFLP	HB	588	135	55	4	275	112	7	9	0.249

For MTHFR rs1801133 polymorphism, AA, AB and BB represent CC, CT and TT, respectively; For MTHFR rs1801131 polymorphism, AA, AB and BB refer to AA, AC and CC. HB = hospital based, HCC = hepatocellular carcinoma, HWE = Hardy-Weinberg Equilibrium, PB = population based, PCR-DHPLC = Polymerase Chain Reaction-Denaturing High Performance Liquid Chromatography, PCR-DNA microarray-based assay = Polymerase Chain Reaction with microarray-based assay, PCR-Illumina Golden Gate platform = Polymerase Chain Reaction based on Illumina Golden Gate platform, PCR-QIAamp DNA Blood Mini Kit = Polymerase Chain Reaction with QIAamp DNA Blood Mini Kit, PCR-RFLP = Polymerase Chain Reaction-Restriction Fragment Length Polymorphism, PCR-SNaPshot = Polymerase Chain Reaction with SNaPshot, TaqMan = Polymerase Chain Reaction with TaqMan probe

* *P* value for Hardy-Weinberg Equilibrium test in controls.

Table 2

Pooled analysis and subgroup analysis of associations between MTHFR polymorphisms and HCC.

Type of analysis	Number of the studies	Allelic genetic model			Dominant genetic model			Recessive genetic model			Heterozygote genetic model			Homozygote genetic model		
		OR [95% CI]	$P_{heterogenity}$/I^2/EM	$P_{meta\text{-}analysis}$	OR [95% CI]	$P_{heterogenity}$/I^2/EM	$P_{meta\text{-}analysis}$	OR [95% CI]	$P_{heterogenity}$/I^2/EM	$P_{meta\text{-}analysis}$	OR[95% CI]	$P_{heterogenity}$/I^2/EM	$P_{meta\text{-}analysis}$	OR [95% CI]	$P_{heterogenity}$/I^2/EM	$P_{meta\text{-}analysis}$
***MTHFR* C677T polymorphism**		T VS C			TT VS TC+CC			TT+TC VS CC			TC VS CC			TT VS CC		
Pooled analysis	20	1.05 [0.95, 1.17]	.000/68/R	.330	1.09 [0.96, 1.25]	.004/52/R	.190	0.98 [0.78, 1.24]	.000/80/R	.880	**1.10 [1.01, 1.20]**	**.17/23/F**	**.040**	1.11 [0.91, 1.35]	.000/60/R	.320
Subgroup analysis																
HWE																
In accordance with HWE	15	1.02 [0.92, 1.13]	.02/48/F	.740	1.09 [0.95, 1.25]	.16/27/F	.240	0.86 [0.66, 1.12]	.000/76/R	.270	1.04 [0.91, 1.18]	.29/15/F	.550	1.10 [0.85, 1.43]	.000/65/R	.480
Departure from HWE	5	1.17 [0.87, 1.59]	.000/74/R	.300	1.12 [0.80, 1.58]	.000/80/R	.500	1.40 [0.84, 2.33]	0.000/88/R	.190	1.21 [0.97, 1.51]	.21/32/F	.090	1.15 [0.85, 1.54]	.14/42/F	.360
Region																
China	12	1.04 [0.94, 1.15]	.002/63/R	.490	**1.11 [1.01, 1.21]**	**.05/44/F**	**.030**	1.09 [0.87, 1.36]	.000/79/R	.470	**1.11 [1.01, 1.22]**	**.22/23/F**	**0.030**	1.13 [0.94, 1.34]	.05/43/F	.190
France	2	0.87 [0.55, 1.39]	.15/51/R	.560	0.91 [0.59, 1.41]	.19/41/F	.670	1.08 [0.58, 2.01]	.79/0/F	.810	1.01 [0.63, 1.62]	.28/13/F	.960	0.66 [0.25, 1.76]	.15/52/R	.410
Italy	3	1.78 [0.77, 4.11]	.000/88/R	.180	1.71 [0.67, 4.33]	.006/81/R	.260	1.33 [0.24, 7.46]	.000/92/R	.750	1.35 [0.88, 2.07]	0.06/64/R	.160	2.45 [0.65, 9.29]	0.002/83/R	.190
South Korea	1	1.04 [0.73, 1.47]	NA	.830	0.93 [0.56, 1.57]	NA	.800	0.71 [0.37, 1.37]	NA	.310	0.87 [0.50, 1.50]	NA	.610	1.16 [0.57, 2.38]	NA	.680
USA	1	0.82 [0.59, 1.15]	NA	.240	0.76 [0.48, 1.20]	NA	.240	0.53 [0.27, 1.05]	NA	.070	0.78 [0.48, 1.26]	NA	.310	0.70 [0.34, 1.45]	NA	.340
Brazil	1	1.07 [0.73, 1.56]	NA	.730	1.11 [0.66, 1.86]	NA	0.710	0.24 [0.10, 0.57]	NA	.001	1.10 [0.64, 1.89]	NA	.730	0.48 [0.22, 1.05]	NA	.070
Enthnicity																
Mongoloid	13	1.04 [0.94, 1.14]	.004/59/R	.460	1.09 [0.95, 1.24]	.06/41/F	.210	1.06 [0.85, 1.32]	.000/78/R	.590	**1.10 [1.00, 1.21]**	**.24/20/F**	**.040**	1.13 [0.95, 1.33]	.08/38/F	.160
Caucasian	7	1.18 [0.82, 1.72]	.000/81/R	.380	1.17 [0.78, 1.77]	.004/69/R	.440	0.84 [0.39, 1.80]	.000/83/R	.660	1.06 [0.84, 1.34]	.14/38/F	.640	1.09 [0.54, 2.23]	.000/78/R	.810
Source of controls																
Hospital Based	18	1.04 [0.92, 1.17]	.000/70/R	.570	1.05 [0.91, 1.22]	.007/51/R	.470	1.01 [0.78, 1.30]	0.000/81/R	.960	1.07 [0.97, 1.17]	.23/19/F	.180	1.08 [0.87, 1.35]	.000/62/R	.490
Population Based	2	**1.21 [1.04, 1.40]**	**.93/0/F**	**.010**	**1.43 [1.10, 1.86]**	**.68/0/F**	**.007**	0.82 [0.53, 1.28]	.11/61/R	.380	**1.40 [1.07, 1.84]**	**.50/0/F**	**.020**	1.36 [0.98, 1.88]	.40/0/F	.070
***MTHFR* A1298C polymorphism**		C VS A			CC+CA VS AA			CC VS CA+AA			CA VS AA			CC VS AA		
Pooled analysis	8	1.07 [0.78, 1.48]	.000/87/R	.680	1.08 [0.84, 1.39]	.001/71/R	.560	0.49 [0.16, 1.46]	.000/91/R	.200	1.03 [0.89, 1.18]	.21/27/F	0.730	0.79 [0.31, 2.01]	.000/87/R	.620
Subgroup analysis																
HWE																
In accordance with HWE	4	1.01 [0.85, 1.21]	.60/0/F	.920	1.25 [1.01, 1.54]	.35/8/F	.040	**0.51 [0.29, 0.92]**	**.79/0/F**	**.020**	1.15 [0.93, 1.42]	.61/0/F	.200	0.62 [0.35, 1.12]	.67/0/F	.110
Departure from HWE	4	1.09 [0.59, 2.03]	.000/94/R	.780	0.94 [0.63, 1.42]	.002/80/R	.770	0.49 [0.07, 3.18]	.000/96/R	0.450	0.94 [0.78, 1.13]	.11/50/R	0.520	0.89 [0.19, 4.08]	.000/94/R	.880
Region																
China	5	0.83 [0.64, 1.09]	.004/74/R	.190	0.98 [0.70, 1.37]	.009/78/R	.890	**0.31 [0.14, 0.65]**	**.03/61/R**	**.002**	0.98 [0.84, 1.16]	.19/35/F	0.850	**0.42 [0.22, 0.78]**	**.13/44/F**	**.006**
South Korea	1	1.27 [0.78, 2.08]	NA	.330	1.46 [0.84, 2.52]	NA	.180	0.20 [0.02, 1.76]	NA	.150	1.58 [0.90, 2.76]	NA	.110	0.46 [0.05, 4.04]	NA	.490
USA	1	1.92 [1.47, 2.52]	NA	.000	1.01 [0.69, 1.47]	NA	.950	5.16 [2.99, 8.90]	NA	.000	0.94 [0.64, 1.40]	NA	.760	3.87 [2.21, 6.79]	NA	.000
Brazil	1	1.74 [1.19, 2.53]	NA	.004	1.65 [0.99, 2.76]	NA	.050	0.54 [0.27, 1.09]	NA	.090	1.33 [0.74, 2.36]	NA	.340	2.75 [1.35, 5.59]	NA	.005
Enthnicity																
Mongoloid	6	0.88 [0.68, 1.14]	.003/73/R	.340	1.03 [0.76, 1.40]	.001/76/R	.850	**0.30 [0.15, 0.58]**	**.06/52/R**	**.000**	1.02 [0.87, 1.19]	.12/42/F	.800	**0.41 [0.24, 0.72]**	**.21/31/F**	**.002**
Caucasian	2	**1.86 [1.49, 2.31]**	**.67/0/F**	**.000**	1.25 [0.77, 2.02]	.13/57/R	.360	1.69 [0.19, 15.39]	.000/96/R	.640	1.05 [0.76, 1.45]	.34/0/F	.780	**3.39 [2.18, 5.27]**	**.45/0/F**	**.000**
Source of Controls																
Hospital Based	7	1.11 [0.76, 1.61]	.000/89/R	.600	1.10 [0.81, 1.50]	.000/75/R	.530	0.58 [0.18, 1.85]	.000/91/R	.360	1.01 [0.86, 1.18]	.15/36/F	.950	0.93 [0.35, 2.48]	.000/87/R	.890
Population Based	1	0.87 [0.67, 1.12]	NA	.280	0.97 [0.73, 1.30]	NA	.850	0.15 [0.05, 0.43]	NA	.000	1.10 [0.81, 1.48]	NA	.540	0.26 [0.09, 0.76]	NA	.010

EM = effect model, F = fixed effect model, HWE = Hardy Weinberg Equilibrium, NA = not available for one study, R = random effect model. Pheterogenity means *P* value for heterogeneity; P meta-analysis means *P* value for meta-analysis.
Significant association with more than one study in each research group was bold and color red.

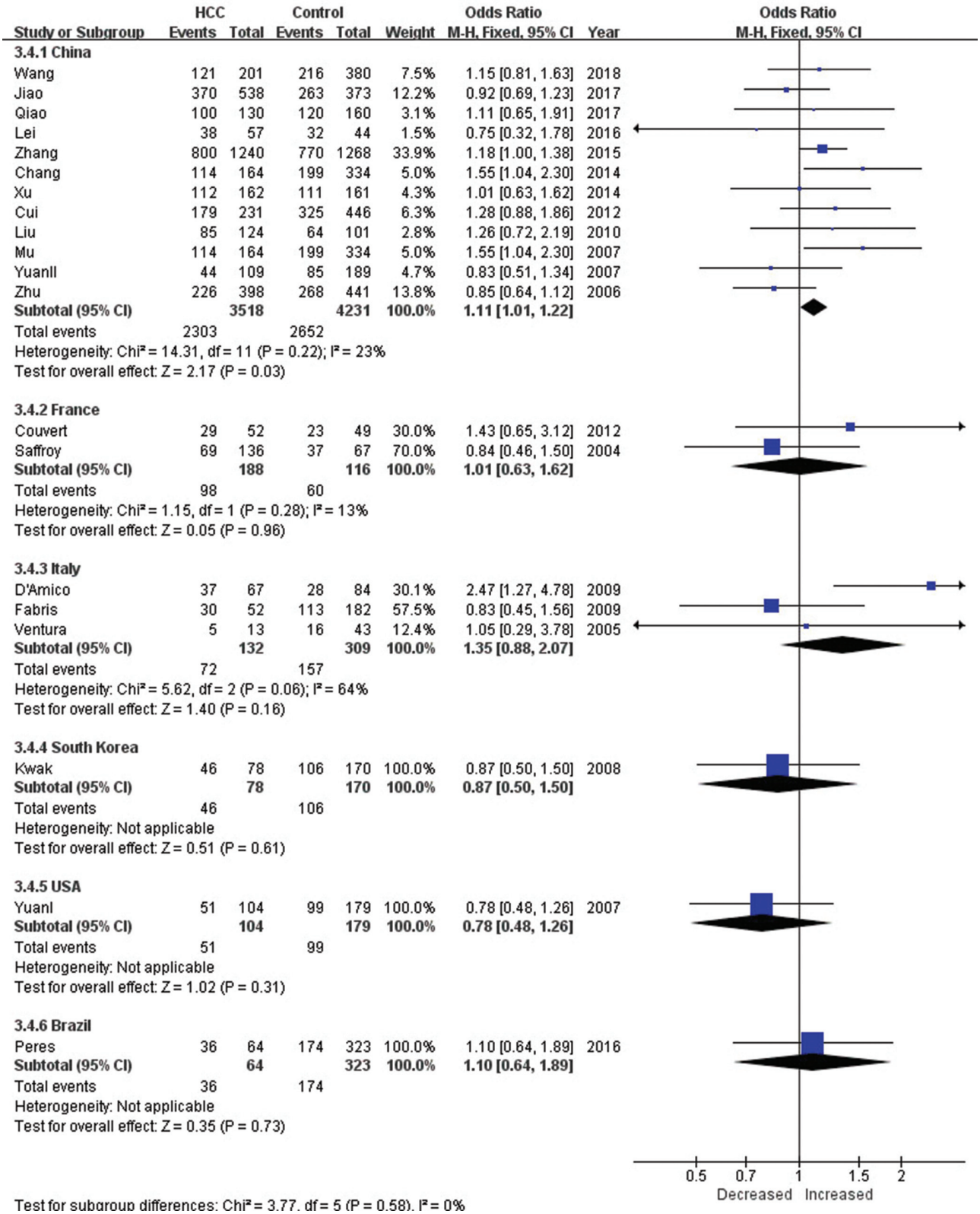

Figure 2. Forest plot of the subgroup analysis of the MTHFR rs1801133 heterozygote genetic model (TC VS CC) and susceptibility to hepatocellular carcinoma in different regions.

China, South Korea, USA and Brazil were recruited. The quality assessment scale was showed in Table S2 (see Table S2, Supplemental Digital Content, which showed the quality assessment scale, http://links.lww.com/MD/G442) and the scores ranged from 6 to 9, which indicated the reliability of our included studies. The PRISMA checklist was attached as Table S3 (see Table S3, Supplemental Digital Content, which showed the PRISMA checklist, http://links.lww.com/MD/G443).

3.2. Meta-analysis results and heterogeneity analysis

Table 2 summarized the pooled and subgroup analysis of associations between the 2 MTHFR SNPs and HCC risk. In the pooled analysis of these 2 polymorphisms, significant association was only detected in heterozygote genetic model (OR = 1.10, 95% CI = [1.01, 1.20], $P_{meta\text{-}analysis} = 0.04$; $P_{Heterogeneity} = 0.17$, $I^2 = 23$) for MTHFR rs1801133 polymorphism (Fig. 1), but for the MTHFR rs1801131 polymorphism, no significant association was found. In order to discover the potential associations and resource of heterogeneity, we conducted a comprehensive subgroup analysis stratified by HWE (In accordance with HWE or departure from HWE), Region (China, France, Italy, South Korea, USA, Brazil), Ethnicity (Mongoloid or Caucasian), and source of controls (Population based or Hospital based).

For the subgroup analysis stratified by HWE, no association was observed for the MTHFR rs1801133 polymorphism; significant association was detected in recessive genetic model (OR = 0.51, 95% CI = [0.29, 0.92], $P_{meta\text{-}analysis} = .02$; $P_{Heterogeneity} = 0.79$, $I^2 = 0$) in the subgroup in accordance with HWE for the MTHFR rs1801131 polymorphism. In the subgroups catalogued by region, significant associations were observed in dominant genetic model (OR = 1.11, 95% CI = [1.01, 1.21], $P_{meta\text{-}analysis} = .03$; $P_{Heterogeneity} = .05$, $I^2 = 44$) and heterozygote genetic model (OR = 1.11, 95% CI = [1.01, 1.22], $P_{meta\text{-}analysis} = .03$; $P_{heterogeneity} = .22$, $I^2 = 23$) in the Chinese subgroup for the MTHFR rs1801133 polymorphism (Fig. 2); as for the MTHFR rs1801131 polymorphism, significant associations were observed in recessive genetic model (OR = 0.31, 95% CI = [0.14, 0.65], $P_{meta\text{-}analysis} = .002$; $P_{Heterogeneity} = .03$, $I^2 = 61$) and homozygote genetic model (OR = 0.36, 95% CI = [0.23, 0.55], $P_{meta\text{-}analysis} = .006$; $P_{heterogeneity} = .13$, $I^2 = 44$) for the Chinese (Fig. 3). For the MTHFR rs1801133 polymorphism, significant association was observed in the heterozygote genetic model (OR = 1.10, 95% CI = [1.00, 1.21], $P_{meta\text{-}analysis} = .04$; $P_{Heterogeneity} = .24$, $I^2 = 20$) in Mongoloid subgroup (Fig. 4); but for the subgroup analysis stratified by ethnicity of the MTHFR rs1801131 polymorphism, significant associations were widely observed [Mongoloid: recessive genetic model (OR = 0.30, 95%

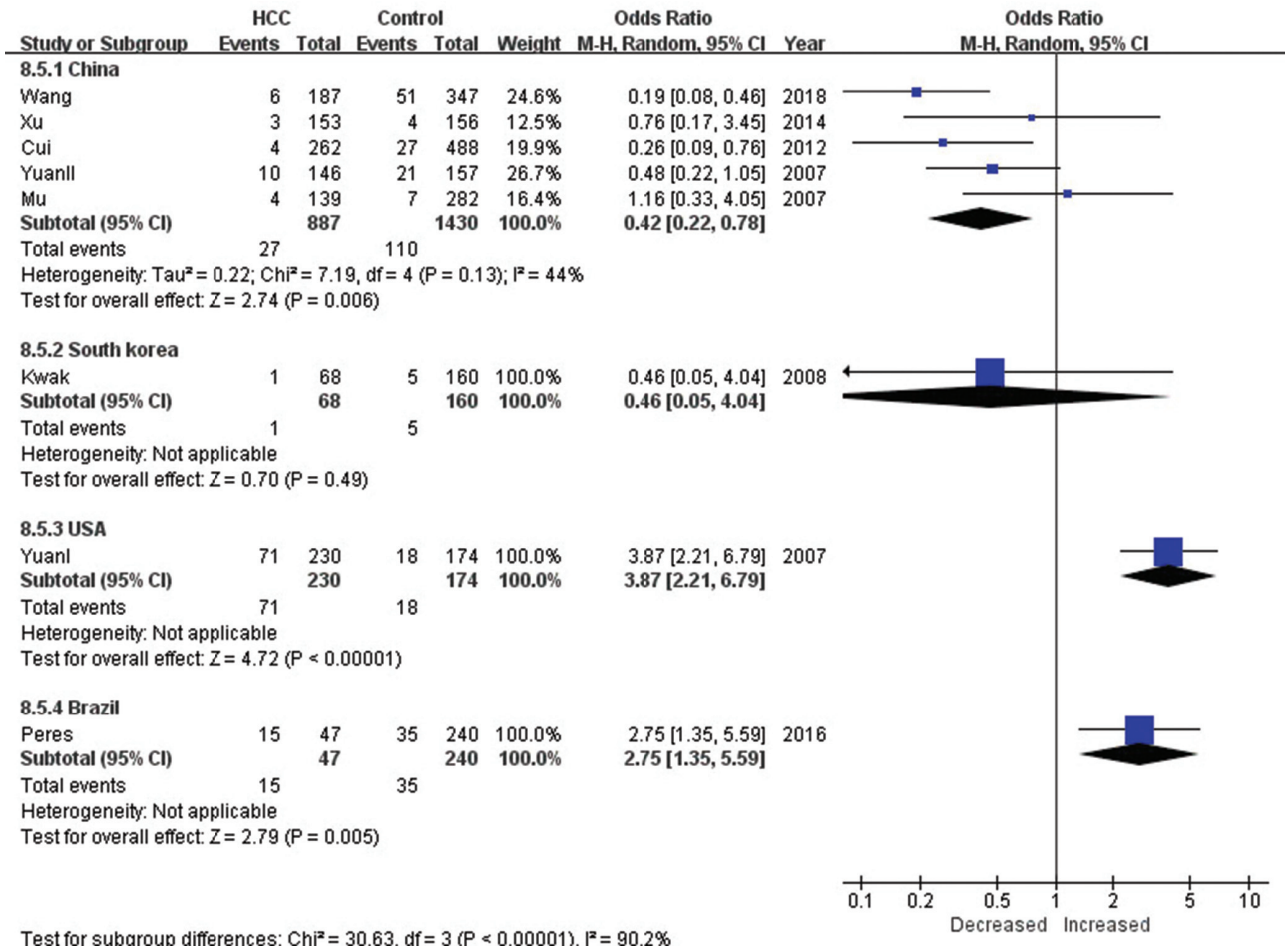

Figure 3. Forest plot of the subgroup analysis of the MTHFR rs1801131 homozygote genetic model (CC VS AA) and susceptibility to hepatocellular carcinoma in different regions.

CI = [0.15, 0.58], $P_{meta\text{-}analysis}$ = .000; $P_{heterogeneity}$ = .06, I^2 = 52), homozygote genetic model (OR = 0.36, 95% CI = [0.24, 0.55], $P_{meta\text{-}analysis}$ = .000; $P_{heterogeneity}$ = .21, I^2 = 31); Caucasian: allelic genetic model (OR = 1.86, 95% CI = [1.49, 2.31], $P_{meta\text{-}analysis}$ = .000; $P_{Heterogeneity}$ = .67, I^2 = 0), homozygote genetic model (OR = 3.47, 95% CI = [2.24, 5.39], $P_{meta\text{-}analysis}$ = .000; $P_{heterogeneity}$ = .45, I^2 = 0)] (Fig. 5). As for the subgroup analysis stratified by source of controls, no association was observed for the rs1801131 polymorphism and significant associations were found in allelic genetic model (OR = 1.21, 95% CI = [1.04, 1.40], $P_{meta\text{-}analysis}$ = .010; $P_{heterogeneity}$ = .93, I^2 = 0), dominant genetic model (OR = 1.43, 95% CI = [1.10, 1.86], $P_{meta\text{-}analysis}$ = .007; $P_{heterogeneity}$ = .68, I^2 = 0) and heterozygote genetic model (OR = 1.40, 95% CI = [1.07, 1.84], $P_{meta\text{-}analysis}$ = .020; $P_{heterogeneity}$ = .50, I^2 = 0) for the MTHFR rs1801133 polymorphism.

3.3. Sensitivity analysis and publication bias

The results of sensitivity analysis indicated that any single study had no significant effect on pooled OR of relationship between the MTHFR rs1801133 and rs1801131 polymorphisms and HCC risk (Fig. 6). Funnel plots for the MTHFR rs1801133 polymorphism under the heterozygote genetic model was symmetrical, implying no significant publication bias (Fig. 7) and the Egger linear regression test (P = .845) also confirmed the negation of publication bias. But for the MTHFR rs1801131 polymorphism, because the number of studies included was less than 10, publication bias could not be assessed.[57]

3.4. Trial sequential analysis

Trial sequential analysis was introduced to evaluate the pooled results of the MTHFR rs1801133 polymorphism and HCC risk under heterozygote genetic model (Fig. 8). Based on the sample size estimation, a sample size of 11259 was required to detect a plausible result for the association of the MTHFR rs1801133 polymorphism. In the present study, a sample size of 9412 has been tested using the heterozygote genetic model (TC VS CC), moreover, the Z curve line only cross the conventional boundary and do not cross the TSA boundary line, indicating that the cumulative evidence is inconclusive and further studies are required to confirm conclusion.

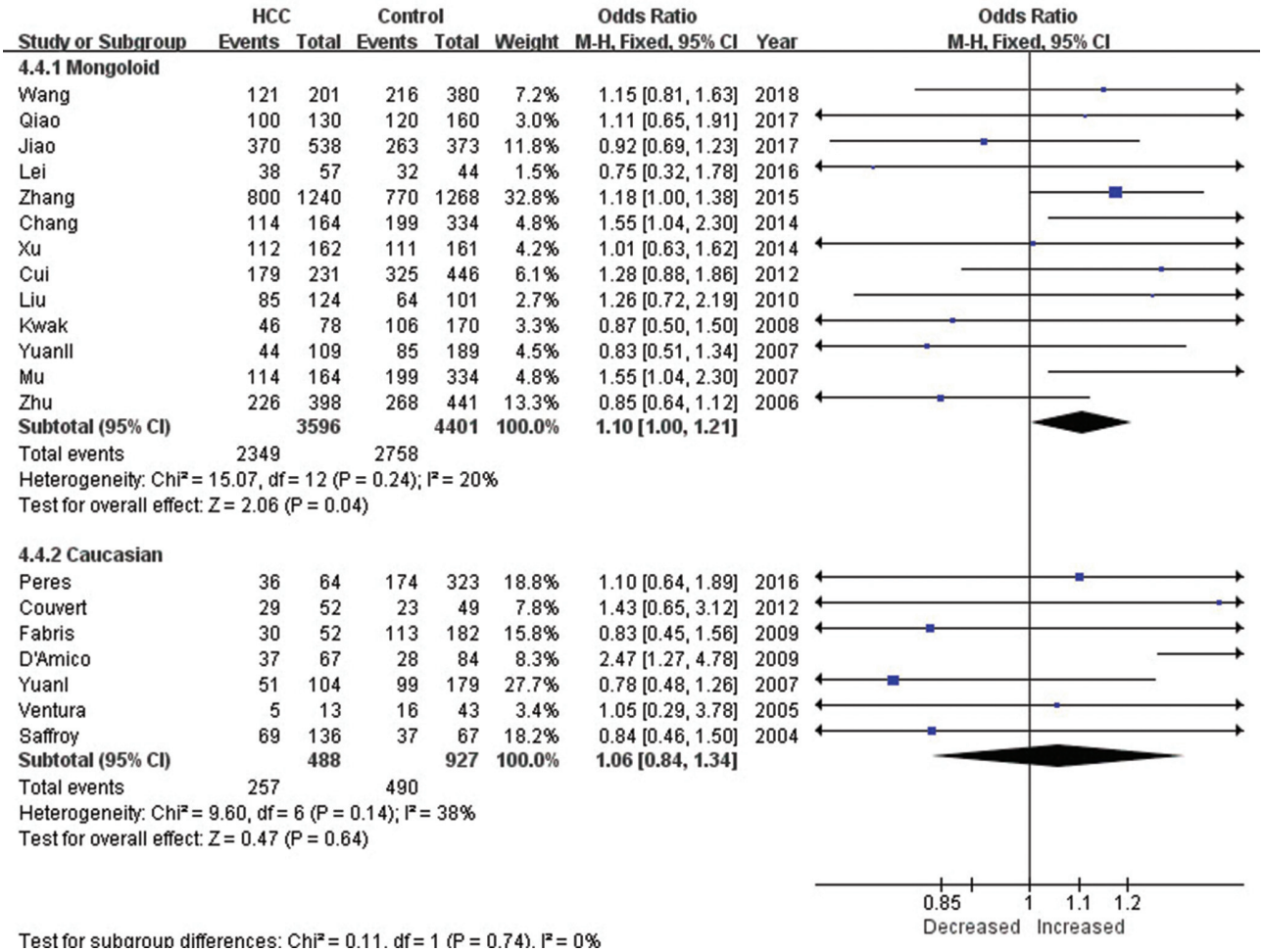

Figure 4. Forest plot of the subgroup analysis of the MTHFR rs1801133 heterozygote genetic model (TC VS CC) and susceptibility to hepatocellular carcinoma in different ethnicities.

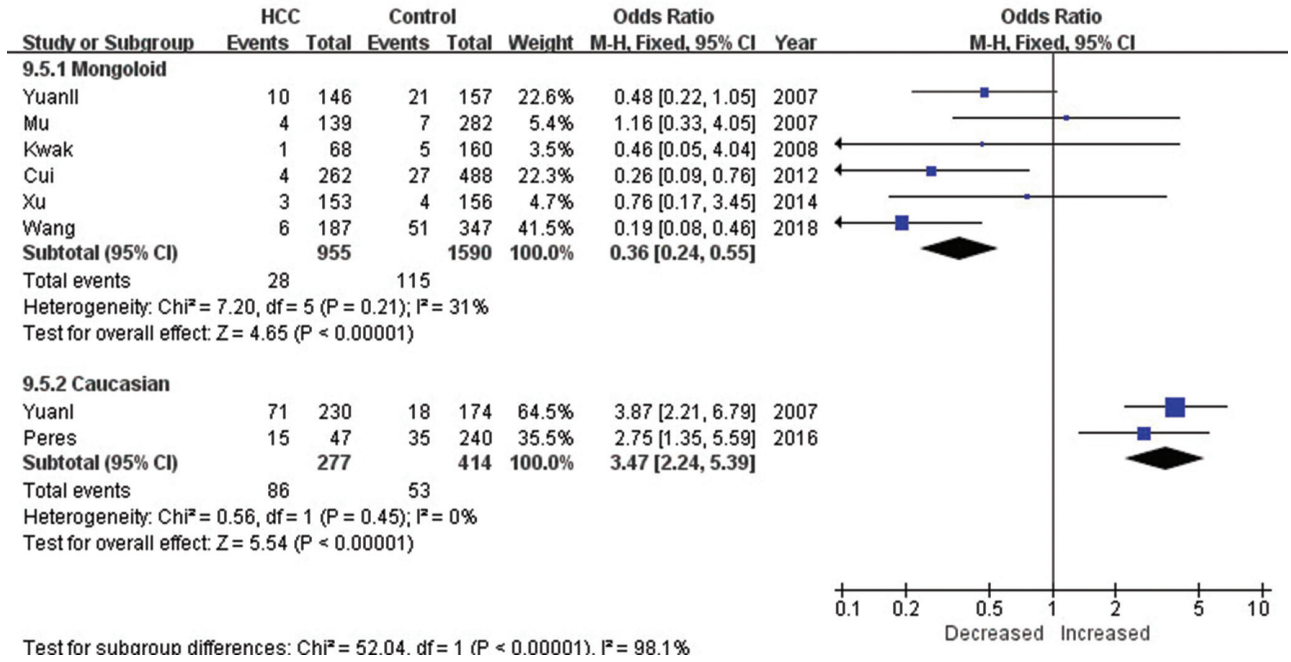

Figure 5. Forest plot of the subgroup analysis of the MTHFR rs1801131 homozygote genetic model (CC VS AA) and susceptibility to hepatocellular carcinoma in different ethnicities.

4. Discussion

Abnormal DNA synthesis and methylation caused by environmental or genetic factors play important role in the occurrence and development of HCC. A lower MTHFR activity will lead to the increased pool of 5,10-methylenetetrahydrofolate for thymidylate synthase and the decreased pool of 5-methyltetrahydrofolate for SAMe, which could favor optimal DNA synthesis, methylation and repair by reducing uracil mis incorporation and double strand breaks of DNA.[58] Functional researches have indicated that subjects with the mutant allele of these 2 polymorphisms showed lower MTHFR enzyme activities.[59,60] Previous meta-analysis reported an increased risk of HCC in the MTHFR rs1801133 polymorphism,[31,61–63] but the small sample size could bias the results, in addition, the influence of studies departure from Hardy Weinberg Equilibrium on the analysis was not discussed in the former studies. As for the MTHRF rs1801131 polymorphism, no association was detected in meta-analysis, but several late case-control studies reported the polymorphism was associated with HCC risk.[13,29] The important biological role of the 2 polymorphisms and the inconsistent conclusions from previous studies draw us to re-evaluate the associations between MTFHR polymorphisms and HCC risk with comprehensive subgroup analysis and trial sequential analysis.

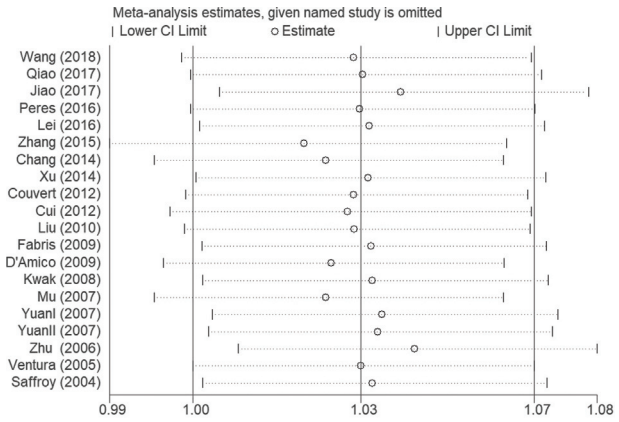

Figure 6. Sensitivity analysis of the pooled analysis of the MTHFR rs1801133 heterozygote genetic model (TC VS CC) and susceptibility to hepatocellular carcinoma.

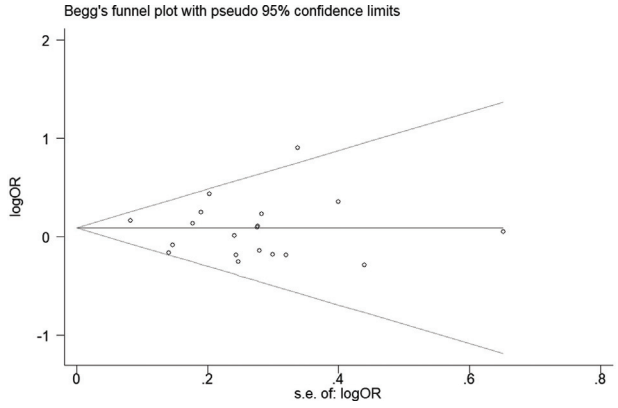

Figure 7. Publication bias of the pooled analysis of the MTHFR rs1801133 heterozygote genetic model (TC VS CC) and susceptibility to hepatocellular carcinoma.

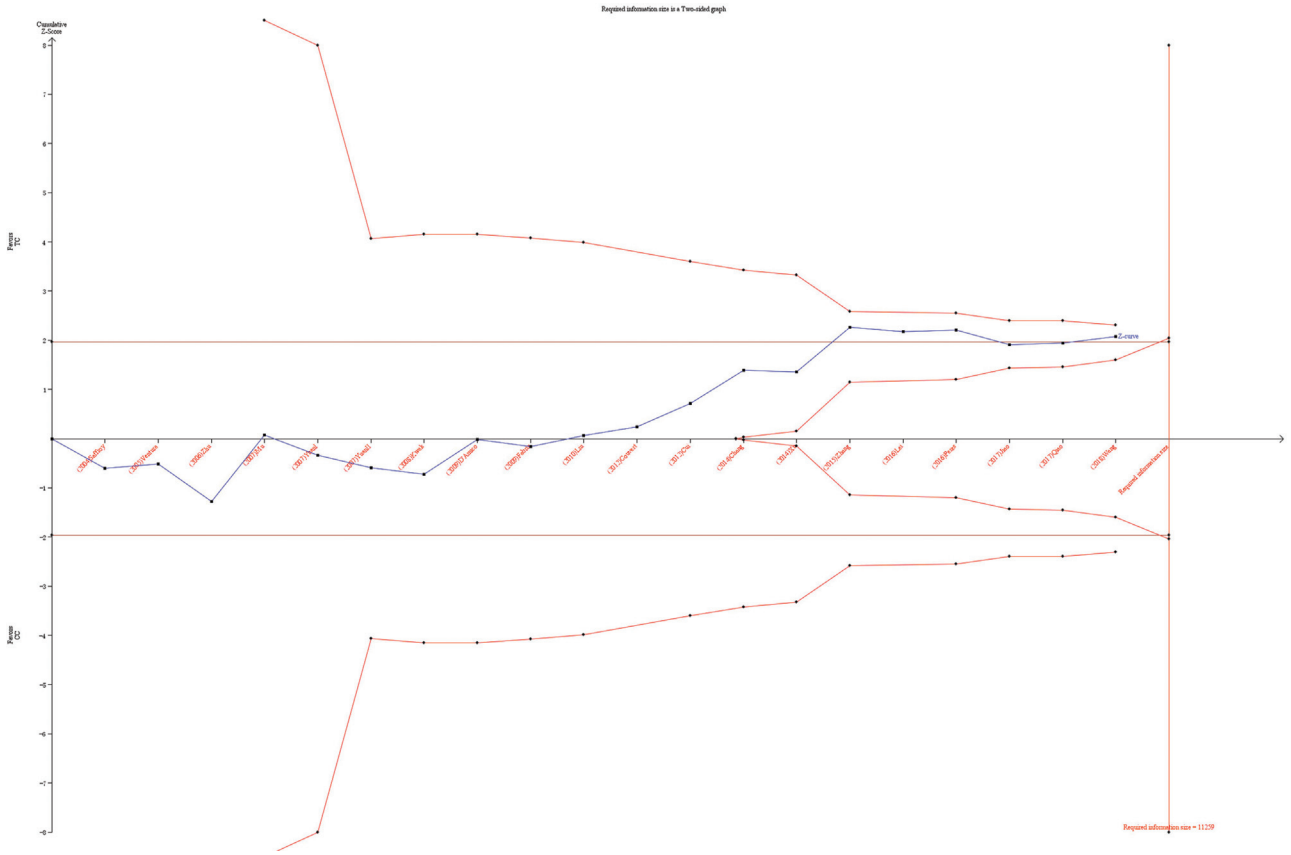

Figure 8. Trial sequential analysis of the pooled analysis of the MTHFR rs1801133 heterozygote genetic model (TC VS CC) and susceptibility to hepatocellular carcinoma.

Our study included nineteen articles, involving 20 studies with 5227 HCC patients and 6688 healthy controls for the MTHFR rs1801133 polymorphism and 8 studies with1714 HCC patients and 2775 healthy controls for the MTHFR rs1801131 polymorphism. The pooled meta-analysis results showed that the MTHFR rs1801133 polymorphism in the heterozygote genetic model was associated with a high risk of developing HCC. It implied the TC genotypes had a 10% increased risk of HCC compared to CC genotypes (OR [95% CI] = 1.10 [1.01, 1.20]). Moreover, the results of sensitivity analysis and publication bias also increased the reliability and stability of the association. However, the TSA results required more further large sample size studies to confirm the association. As for the MTHFR rs1801131 polymorphism, no association was discovered.

The differences in the genetic equilibrium of control group, region, ethnicity, source of controls may have an influence on the risk of developing HCC in a way of gene-environment interaction. Hence, we performed a comprehensive subgroup analysis based on the differences mentioned above. To test the Hardy-Weinberg Equilibrium in the control group is essential to reflect the homogeneity of selected population and reduce the bias in enroll research subjects. In the subgroup in accordance with HWE, a decreased risk of the MTHFR rs1801131 polymorphism under the recessive genetic model was observed (OR [95% CI] = 0.51 [0.29, 0.92]), indicating the CC genotype had a 49% decreased risk of HCC compared to CA/AA genotypes. Hospital based and Population based are the 2 main source of controls, the advantages of low selection bias and more randomization in Population based control group could generate more reliable and solid results. In the subgroup analysis stratified by source of controls, extensive increased risks of HCC in the MTHFR rs1801133 polymorphism under allelic (OR [95% CI] = 1.21 [1.04, 1.40]), dominant (OR [95% CI] = 1.43 [1.10, 1.86]), heterozygote (OR [95% CI] = 1.40 [1.07, 1.84]) genetic model were observed.

Geography information is an important environment variable for gene-environment interaction. Significant associations were observed for these 2 polymorphisms in Chinese group. The MTHFR rs1801133 polymorphism was associated with a high risk of HCC under dominant genetic model (OR [95% CI] = 1.11 [1.01, 1.21]) and heterozygote genetic model (OR [95% CI] = 1.11 [1.01, 1.22]). A decreased risk of HCC for MTHFR rs1801131 polymorphism was detected in recessive (OR [95% CI] = 0.31 [0.14, 0.65]) and homozygote (OR [95% CI] = 0.36 [0.24, 0.55]) genetic model. In addition, the same increased risk of HCC for the MTHFR rs1801133 polymorphism under the heterozygote genetic model was observed in Mongoloid population. But for the MTHFR rs1801131 polymorphism, interesting results were emerged. The decreased risk of HCC was detected in Mongoloid under the recessive (OR [95% CI] = 0.30 [0.15, 0.58]) and homozygote genetic model (OR [95% CI] = 0.36 [0.23, 0.55]). But the increased risk of HCC was observed in Caucasian under the allelic (OR [95% CI] = 1.86 [1.49, 2.31])

and homozygote (OR [95% CI] = 3.47 [2.24, 5.39]) genetic model. As a brief summary, in Mongoloid especially in Chinese, an increased risk of HCC for the MTHFR rs1801133 and a decreased risk of HCC for the MTHFR rs1801131 were observed, nevertheless, an increased risk of HCC for the MTHFR rs1801131 in Caucasian was discovered.

The contrary risk associations in the Mongoloid and Caucasian populations of the MTHFR rs1801131 polymorphism arouse our great interests. After literature intensive reading, we found there are 2 ways of changed MTHFR enzyme activity on the HCC risk:

1. the reduced MTFHR enzymatic activity would result in reductive conversion of 5,10-methylenetetrahydrofolate into 5-methylenetetrahydrofolate, next, a decreased level of S-adenosylmethionine lead to down-regulated DNA methylation and an increased risk of HCC occurrence;[64]
2. the reduced activity of MTFHR contribute to an accumulation of 5,10-methylenetetrahydrofolate, resulting in a lower dUMP/dTMP ratio, reduce the incidence of the incorrect incorporation of uracil into the DNA and double-strand DNA breaks, which can strengthen the ability of the DNA and finally lead to a lower HCC risk.[65,66]

We found the mutant 1298C allele could decrease the risk of HCC in Chinese and Mongoloid population, and the epidemic study reported that most cases of HCC occur in Asian,[67] particularly in East Asia with a very high incidence (over 20 cases/100000 population), which was a proof to our results. Anyway, larger studies are required to validate the associations.

Several limitations should be acknowledged in the present meta-analysis. Firstly, associations in relative small sample size in some subgroups should be interpreted with cautions such as the increased risk of HCC in Caucasian for the MTHFR rs1801131 polymorphism, the only 1 study in some subgroups (Brazil, South Korea, USA, Hospital based for MTHFR rs1801131 polymorphism); secondly, only English and Chinese literatures were retrieved, and missing of relevant studies in other language might bias our results; thirdly, for the type of our research (meta-analysis), the unreasonable data and bias in original studies could be the potential confounding factors; at last, the genotyping method were not uniform and could have an influence on the deviation of outcomes. All above, further studies with larger sample size from different regions and ethnicities are required to provide a more accurate association.

In conclusion, this meta-analysis indicates the TC genotype of the MTHFR rs1801133 polymorphism is associated with an increased risk of hepatocellular carcinoma (HCC) risk, in addition, the MTHFR rs1801133 polymorphism was associated with an increased risk of HCC in Mongoloid population especially in Chinese. As for the MTHFR rs1801131 polymorphism, increased HCC risk was observed in Caucasian population, and decreased risk of HCC was remarkably discovered in homozygous mutant CC genotypes in Mongoloid and Chinese subgroups. In the future, larger well-designed studies are warranted to verify these results.

Author contributions

Conceptualization: Lang Li.
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Investigation: Miaomiao Ma, Yan Yan.
Methodology: Miaomiao Ma, Xiaojun Guo, Yan Yan, Lang Li.
Project administration: Lang Li.
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Supervision: Binfeng Wang, Lang Li.
Validation: Binfeng Wang, Miaomiao Ma, Lang Li.
Visualization: Binfeng Wang, Lang Li.
Writing – original draft: Binfeng Wang, Lang Li.
Writing – review & editing: Binfeng Wang, Miaomiao Ma, Xiaojun Guo, Yan Yan, Lang Li.

References

[1] Granito A, Bolondi L. Non-transplant therapies for patients with hepatocellular carcinoma and Child-Pugh-Turcotte class B cirrhosis. Lancet Oncol 2017;18:e101–12.
[2] Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR. A global view of hepatocellular carcinoma: trends, risk, prevention and management. Nat Rev Gastroenterol Hepatol 2019;16:589–604.
[3] Kulik L, El-Serag HB. Epidemiology and management of hepatocellular carcinoma. Gastroenterology 2019;156: 477-491.e471.
[4] Thorsson V, Gibbs DL, Brown SD, et al. The immune landscape of cancer. Immunity 2018;48: 812-830.e814.
[5] Forner A, Reig M, Bruix J. Hepatocellular carcinoma. Lancet 2018;391:1301–14.
[6] Janevska D, Chaloska-Ivanova V, Janevski V. Hepatocellular carcinoma: risk factors;1; diagnosis and treatment. Open Access Macedonian J Med Sci 2015;3:732.
[7] Hack SP, Spahn J, Chen M, et al. IMbrave 050: a Phase III trial of atezolizumab plus bevacizumab in high-risk hepatocellular carcinoma after curative resection or ablation. Future Oncol 2020;16:975–89.
[8] Liu X, Li M, Wang X, et al. Effects of adjuvant traditional Chinese medicine therapy on long-term survival in patients with hepatocellular carcinoma. Phytomedicine 2019;62:152930.
[9] Haines K, Sarabia SF, Alvarez KR, et al. Characterization of pediatric hepatocellular carcinoma reveals genomic heterogeneity and diverse signaling pathway activation. Pediatric Blood Cancer 2019;66:e27745.
[10] Lin W, Chen J, Zhu B, Xu X, Dong Z. Role of toll-like receptors gene polymorphism in hepatocellular carcinoma. J Receptor Signal Transduction Res 2014;34:345–7.
[11] Xie Q, Chen Z, Xia L, Zhao Q, Yu H, Yang Z. Correlations of PD-L1 gene polymorphisms with susceptibility and prognosis in hepatocellular carcinoma in a Chinese Han population. Gene 2018;674:188–94.
[12] Wang B, Hsu CJ, Lee HL, et al. Impact of matrix metalloproteinase-11 gene polymorphisms upon the development and progression of hepatocellular carcinoma. Int J Med Sci 2018;15:653–8.
[13] Wang C, Xie H, Lu D, et al. The MTHFR polymorphism affect the susceptibility of HCC and the prognosis of HCC liver transplantation. Clin Trans Oncol 2018;20:448–56.
[14] Sah S, Lahry K, Talwar C, Singh S, Varshney U. Monomeric NADH-oxidizing methylenetetrahydrofolate reductases from mycobacterium smegmatis lack flavin coenzyme. J Bacteriol 2020;202:e00709–19.
[15] Kaur R, Correa ARE, Thakur S, Kabra M, Gupta N. Methylene tetrahydrofolate reductase deficiency. Indian J Pediatr 2020;87:951–3.
[16] Frikha R. Assessment of the relationship between methylenetetrahydrofolate reductase polymorphism and acute lymphoblastic leukemia: evidence from an updated meta-analysis. J Oncol Pharm Pract 2020;26:1598–610.
[17] Ahmed SF, Ali MM, Kheiri S, Elzaki SEG, Adam I. Association of methylenetetrahydrofolate reductase C677T and reduced-f carrier-1 G80A gene polymorphism with preeclampsia in Sudanese women. Hypertens Pregnancy 2020;39:77–81.
[18] Wan L, Li Y, Zhang Z, Sun Z, He Y, Li R. Methylenetetrahydrofolate reductase and psychiatric diseases. Transl Psychiatry 2018;8:242.
[19] Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10:111–3.
[20] van der Put NM, Gabreels F, Stevens EM, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am J Hum Genet 1998;62:1044–51.
[21] Ventura P, Venturelli G, Marcacci M, et al. Hyperhomocysteinemia and MTHFR C677T polymorphism in patients with portal vein thrombosis complicating liver cirrhosis. Thromb Res 2016;141:189–95.

[22] Gemmati D, Ongaro A, Tognazzo S, et al. Methylenetetrahydrofolate reductase C677T and A1298C gene variants in adult non-Hodgkin's lymphoma patients: association with toxicity and survival. Haematologica 2007;92:478–85.
[23] Petrone I, Bernardo PS, Dos Santos EC, Abdelhay E. MTHFR C677T and A1298C polymorphisms in breast cancer, gliomas and gastric cancer: a review. Genes (Basel) 2021;12:587.
[24] Liu CT, Karasik D, Xu H, et al. Genetic variants modify the associations of concentrations of methylmalonic acid, vitamin B-12, vitamin B-6, and folate with bone mineral density. Am J Clin Nut 2021;114:578–87.
[25] Kuroda K, Horikawa T, Gekka Y, et al. Effects of periconceptional multivitamin supplementation on folate and homocysteine levels depending on genetic variants of methyltetrahydrofolate reductase in infertile Japanese Women. Nutrients 2021;13:1381.
[26] Jiang Y, Xiao X, Wen Y, et al. Genetic effect of MTHFR C677T, A1298C, and A1793G polymorphisms on the age at onset, plasma homocysteine, and white matter lesions in Alzheimer's disease in the Chinese population. Aging (Albany NY) 2021;13:11352–62.
[27] Bjørklund G, Peana M, Dadar M, et al. The role of B vitamins in stroke prevention. Crit Rev Food Sci Nutr 2021;1–14.
[28] Aguilar-Lacasaña S, López-Flores I, González-Alzaga B, et al. Methylenetetrahydrofolate reductase (MTHFR) gene polymorphism and infant's anthropometry at birth. Nutrients 2021;13:831.
[29] Peres NP, Galbiatti-Dias AL, Castanhole-Nunes MM, et al. Polymorphisms of folate metabolism genes in patients with cirrhosis and hepatocellular carcinoma. World J Hepatol 2016;8:1234–43.
[30] Zhang H, Li G, Zhang Z. Association between MTHFR A1298C polymorphism and hepatocellular carcinoma risk. Int J Clin Exp Med 2015;8:9135–41.
[31] Sun H, Han B, Zhai H, Cheng X, Ma K. Significant association between MTHFR C677T polymorphism and hepatocellular carcinoma risk: a meta-analysis. Tumour Biol 2014;35:189–93.
[32] Qiao K, Zhang S, Trieu C, et al. Genetic polymorphism of MTHFR C677T influences susceptibility to HBV-related hepatocellular carcinoma in a Chinese population: a case-control study. Clin Laboratory 2017;63:787–95.
[33] Jiao X, Luo Y, Yang B, et al. The MTHFR C677T mutation is not a risk factor recognized for HBV-related HCC in a population with a high prevalence of this genetic marker. Infection, Genetics Evolution 2017;49:66–72.
[34] Zhang H, Liu C, Han YC, et al. Genetic variations in the one-carbon metabolism pathway genes and susceptibility to hepatocellular carcinoma risk: a case-control study. Tumour Biol 2015;36:997–1002.
[35] Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Int J Surg 2010;8:336–41.
[36] Sun Y, Chen J, Li H, Jiang J, Chen S. Steroid injection and nonsteroidal anti-inflammatory agents for shoulder pain: a PRISMA systematic review and meta-analysis of randomized controlled trials. Medicine 2015;94:e2216.
[37] Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 2010;25:603–5.
[38] Hedges LV, Pigott TD. The power of statistical tests in meta-analysis. Psychological Methods 2001;6:203–17.
[39] Turner RM, Bird SM, Higgins JP. The impact of study size on meta-analyses: examination of underpowered studies in Cochrane reviews. PloS One 2013;8:e59202.
[40] Mandal RK, Dar SA, Jawed A, et al. Impact of LMP7 (rs2071543) gene polymorphism in increasing cancer risk: evidence from a meta-analysis and trial sequential analysis. Oncotarget 2018;9:6572–85.
[41] Ma M, Tao L, Liu A, et al. Macrophage migration inhibitory factor-794 CATT microsatellite polymorphism and risk of tuberculosis: a meta-analysis. Biosci Rep 2018;38:BSR20171626.
[42] Kanu JS, Qiu S, Cheng Y, et al. Associations between three common single nucleotide polymorphisms (rs266729, rs2241766, and rs1501299) of ADIPOQ and cardiovascular disease: a meta-analysis. Lipids Health Dis 2018;17:126.
[43] Chang SC, Chang PY, Butler B, et al. Single nucleotide polymorphisms of one-carbon metabolism and cancers of the esophagus, stomach, and liver in a Chinese population. PloS One 2014;9:e109235.
[44] Cui LH, Song Y, Si H, et al. Folate metabolism-related gene polymorphisms and susceptibility to primary liver cancer in North China. Medical Oncology (Northwood, London, England) 2012;29:1837–42.
[45] Couvert P, Carrie A, Tezenas du Montcel S, et al. Insulin-like growth factor 2 gene methylation in peripheral blood mononuclear cells of patients with hepatitis C related cirrhosis or hepatocellular carcinoma. Clin Res Hepatol Gastroenterol 2012;36:345–51.
[46] Fabris C, Toniutto P, Falleti E, et al. MTHFR C677T polymorphism and risk of HCC in patients with liver cirrhosis: role of male gender and alcohol consumption. Alcoholism, Clin Exp Res 2009;33:102–7.
[47] D'Amico M, Pasta L, Sammarco P. MTHFR C677TT, PAI1 4G-4G, V Leiden Q506, and prothrombin G20210A in hepatocellular carcinoma with and without portal vein thrombosis. J Thromb Thrombolysis 2009;28:70–3.
[48] Kwak SY, Kim UK, Cho HJ, et al. Methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) gene polymorphisms as risk factors for hepatocellular carcinoma in a Korean population. Anticancer Res 2008;28(5a):2807–11.
[49] Yuan JM, Lu SC, Van Den Berg D, et al. Genetic polymorphisms in the methylenetetrahydrofolate reductase and thymidylate synthase genes and risk of hepatocellular carcinoma. Hepatology (Baltimore, Md) 2007;46:749–58.
[50] Mu LN, Cao W, Zhang ZF, et al. Methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms and the risk of primary hepatocellular carcinoma (HCC) in a Chinese population. Cancer Causes Control 2007;18:665–75.
[51] Zhu ZZ, Cong WM, Liu SF, Xian ZH, Wu WQ. A study on the association of MTHFR C677T polymorphism with genetic susceptibility to hepatocellular carcinoma. Zhonghua gan zang bing za zhi = Zhonghua ganzangbing zazhi = Chinese journal of hepatology 2006;14:196–8.
[52] Ventura P, Rosa MC, Abbati G, et al. Hyperhomocysteinaemia in chronic liver diseases: role of disease stage, vitamin status and methylenetetrahydrofolate reductase genetics. Liver Int 2005;25:49–56.
[53] Saffroy R, Pham P, Chiappini F, et al. The MTHFR 677C > T polymorphism is associated with an increased risk of hepatocellular carcinoma in patients with alcoholic cirrhosis. Carcinogenesis 2004;25:1443–8.
[54] Lei Lei, Li Xu, He Jianhua, Li Lei. Study on the interaction between MTHFR C677T polymorphism and alcohol consumption in liver cancer in the population of Enshi. Hebei Yiyao 2016;3339-3340+3343.
[55] Xu Mengjia, Gu Zhaoya, Zhao Jianyuan, Wang Hongyan, Liu Jie, Chen Ying. Genetic polymorphisms of key enzymes in folate metabolism pathway and liver cancer susceptibility analysis. J Fudan Univers 2014;716–23.
[56] Liu Juanjuan , Gao Yingtang , Du Zhi , et al. The relationship between C677T polymorphism of methylenetetrahydrofolate reductase gene and disease outcome after HBV infection. World Chin J Digestion 2010;1555–62.
[57] Song F, Eastwood AJ, Gilbody S, Duley L, Sutton AJ. Publication and related biases. Health Technol Assess 2000;4:1–115.
[58] Baik E, Sanchez DL, Turner PA, Mach KJ, Field CB, Benson SM. Geospatial analysis of near-term potential for carbon-negative bioenergy in the United States. Proc Natl Acad Sci U S A 2018;115:3290–5.
[59] Weisberg I, Tran P, Christensen B, Sibani S, Rozen R. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol Genet Metab 1998;64:169–72.
[60] Chango A, Boisson F, Barbe F, et al. The effect of 677C–>T and 1298A–>C mutations on plasma homocysteine and 5,10-methylenetetrahydrofolate reductase activity in healthy subjects. Br J Nut 2000;83:593–6.
[61] Qi YH, Yao LP, Cui GB, et al. Meta-analysis of MTHFR C677T and A1298C gene polymorphisms: association with the risk of hepatocellular carcinoma. Clin Res Hepatol Gastroenterol 2014;38:172–80.
[62] Qi X, Sun X, Xu J, Wang Z, Zhang J, Peng Z. Associations between methylenetetrahydrofolate reductase polymorphisms and hepatocellular carcinoma risk in Chinese population. Tumour Biol 2014;35:1757–62.
[63] Lv L, Wang P, Sun B, Chen G. The polymorphism of methylenetetrahydrofolate reductase C677T but not A1298C contributes to gastric cancer. Tumour Biol 2014;35:227–37.
[64] Stern LL, Mason JB, Selhub J, Choi SW. Genomic DNA hypomethylation, a characteristic of most cancers, is present in peripheral leukocytes of individuals who are homozygous for the C677T polymorphism in the

methylenetetrahydrofolate reductase gene. Cancer Epidemiol Biomarkers Prev 2000;9:849–53.
[65] Blount BC, Mack MM, Wehr CM, et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proceedings National Academy Sci United States Am 1997;94:3290–5.
[66] Machover D, Zittoun J, Saffroy R, et al. Treatment of cancer cells with methioninase produces DNA hypomethylation and increases DNA synthesis. Cancer Res 2002;62:4685–9.
[67] Gomaa AI, Khan SA, Toledano MB, Waked I, Taylor-Robinson SD. Hepatocellular carcinoma: epidemiology, risk factors and pathogenesis. World J Gastroenterol 2008;14:4300–8.