Associations between Methylenetetrahydrofolate Reductase (MTHFR) Polymorphisms and Non-Alcoholic Fatty Liver Disease (NAFLD) Risk: A Meta-Analysis

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Abstract

Background

C677T and A1298C are the most common allelic variants of Methylenetetrahydrofolate Reductase (MTHFR) gene. The association between MTHFR polymorphisms and the occurrence of non-alcoholic fatty liver disease (NAFLD) remains controversial. This study was thus performed to examine whether MTHFR mutations are associated with the susceptibility to NAFLD.

Methods

A first meta-analysis on the association between the MTHFR polymorphisms and NAFLD risks was carried out via Review Manager 5.0 and Stata/SE 12.0 software. The on-line databases, such as PubMed, EMBASE, CENTRAL, WOS, Scopus and EBSCOhost (updated to April 1st, 2016), were searched for eligible case-control studies. The odd ratio (OR), 95% confidence interval (CI) and P value were calculated through Mantel-Haenszel statistics under random- or fixed-effect model.

Results

Eight articles (785 cases and 1188 controls) contributed data to the current meta-analysis. For C677T, increased NAFLD risks were observed in case group under homozygote model (T/T vs C/C, OR = 1.49, 95% CI = 1.03~2.15, P = 0.04) and recessive model (T/T vs C/C+C/T, OR = 1.42, 95% CI = 1.07~1.88, P = 0.02), but not the other genetics models, compared with control group. For A1298C, significantly increased NAFLD risks were detected in allele model (C vs A, OR = 1.53, 95% CI = 1.13~2.07, P = 0.006), homozygote model (C/C vs A/A, OR = 2.81, 95% CI = 1.63~4.85, P = 0.0002), dominant model (A/C+C/C vs A/A, OR = 1.60,
95% CI = 1.06~2.41, \( P = 0.03 \) and recessive model (C/C vs A/A+A/C, \( \text{OR} = 2.08, 95\% \text{ CI} = 1.45~3.00, P<0.0001 \), but not heterozygote model.

**Conclusion**

T/T genotype of MTHFR C677T polymorphism and C/C genotype of MTHFR A1298C are more likely to be associated with the susceptibility to NAFLD.

**Introduction**

Human Methylene tetrahydrofolate Reductase (MTHFR) gene is located at chromosome 1p36.3 and contains 11 exons [1, 2]. As a kind of folate-metabolizing enzyme, MTHFR protein is essential for the methylation of homocysteine (Hcy) to methionine, through catalyzing the irreversible reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate [3–6]. The abnormality of MTHFR structure or function can take part in the occurrence of Hyperhomocysteinemia [5–7]. Two polymorphic variants, including C677T (rs1801133) and A1298C (rs1801131), have been identified in MTHFR gene [8–11].

Non-alcoholic fatty liver disease (NAFLD), the most common chronic liver disease, is the hepatic manifestation of the metabolic syndrome without a history of excess alcohol consumption [12–14]. The hepatic pathology of NAFLD mainly consists of simple fatty liver, non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis [15–18]. And NASH was characterized by hepatocellular injury and inflammation [15–18]. The polymorphisms of several genes, such as Patatin-like phospholipase domain-containing 3 (PNPLA3), leptin receptor (LEPR) and MTHFR, were reported to be involved in the genetic susceptibility to NAFLD [19–21]. For MTHFR gene, conflicting results regarding its potential correlation with NAFLD were reported [22–30]. Here, we focus on the polymorphisms of human MTHFR and assessed its genetic association with NAFLD risks via a meta-analysis, a very powerful tool for integrating and analyzing the conflicting data from different studies [31].

To our knowledge, no meta-analysis on the association of MTHFR genetic variants and overall NAFLD risks has been reported. Hence, we first carried out a meta-analysis to investigate the relationship between MTHFR polymorphisms (C677T and A1298C) and susceptibility to NAFLD. Our finding showed that both C677T and A1298C polymorphisms of MTHFR gene might positively correlate to the risks of NAFLD.

**Methods**

**Searching strategy**

A computerized literature search was performed from the electronic databases, including PubMed, Excerpta Medica Database (EMBASE), Cochrane Central Register of Controlled Trials (CENTRAL), Web of Science (WOS), China National Knowledge Infrastructure (CNKI)/WANFANG, Scopus and EBSCOhost in April 1st, 2016. There was no language or region restriction. The combinations of following keywords were used: “Methylene tetrahydrofolate Reductase (NADPH)” or “Methylene-THF Reductase (NADPH)” or “MTHFR” or “Methylenetetrahydrofolate Reductase”; “Non-alcoholic Fatty Liver Disease” or “NAFLD” or “Fatty Liver, Nonalcoholic” or “Livers, Nonalcoholic Fatty” or “Nonalcoholic Fatty Livers” or “Nonalcoholic Steatohepatitis” or “Nonalcoholic Steatohepatitides” or “Steatohepatitides, Nonalcoholic” or “Steatohepatitis, Nonalcoholic”; “Polymorphism, Genetic” or “Genetic Polymorphisms” or
“Genetic Polymorphism” or “Polymorphism (Genetics)” or "Polymorphisms, Genetic”. The full details of databases searching terms were also provided (S1 Text).

Inclusion and exclusion criteria
The eligible case-control studies were identified according to the following inclusion and exclusion criteria. Inclusion criteria: 1) The data on the association between MTHFR polymorphisms and susceptibility to NAFLD was provided; 2) The individual genotype frequencies for MTHFR polymorphisms could be extracted. Exclusion criteria: 1) duplicated studies; 2) reviews or books; 3) non-clinical data; 4) other genes; 5) non-NAFLD diseases; 6) case, trial, or non-polymorphism; 7) meeting/conference abstracts; 8) unavailable data.

Data extraction strategy
Data was extracted from qualified articles independently by the authors (MYS LZ SLS JNL) using the same reporting form. The controversial evaluations were resolved through discussion. If the data was unavailable, an attempt was made to contact corresponding author to request missing data via E-mail. The following information was extracted: mutation site, first author, year of publication, country, ethnicity, sample sizes in case and control group, source of control, genotyping method, gender and age in case group, disease group, allele and genotype frequencies in each group, The \( \chi^2 \) and \( P \) value of Hardy-Weinberg Equilibrium (HWE) test in control group. HWE value was calculated by chi-squared test and \( P \) value less than 0.05 was considered a departure from HWE.

Statistical analysis
The \( P \) value, odd radio (OR) and corresponding 95% confidence interval (CI) were calculated by Mantel-Haenszel statistics under the allele, homozygote, heterozygote, dominant or recessive models. \( P \) value <0.05 was considered statistically significant association between C677T and A1298C polymorphisms of MTHFR and NAFLD risks. \( \chi^2 \)-based Q statistic and I\(^2\) test were applied to analyze the overall heterogeneities. When I\(^2\) values < 25% or \( P \) value of heterogeneity >0.10, a fixed-effect model was selected for Mantel-Haenszel statistics. Otherwise, a random-effect model was used [32–35]. When significant heterogeneity existed, sensitivity analysis was also performed to analyze the study that influenced homogeneity of the included studies. The potential publication bias was evaluated by Begg’s funnel plot with pseudo 95% confidence limits [36]. Statistical analyses were conducted by Review Manager Version 5.0 (The Nordic Cochrane Centre, The Cochrane Collaboration, Denmark) and Stata/SE 12.0 (StataCorp, College Station, USA) software.

Results
Study inclusion and characteristics
We searched the on-line electronic databases, including PubMed, EMBASE, CENTRAL, WOS, CNKI/WANFANG, Scopus and EBSCOhost (updated to April 1\(^4\), 2016), to obtain the eligible case-control studies. Flow chart of studies selection in meta-analysis was shown in Fig 1.

Possibly relevant articles of 221 were obtained from the electronic databases, including PubMed (n = 10), EMBASE (n = 29), CENTRAL (n = 0), WOS (n = 24), CNKI/WANFANG (n = 3), Scopus (n = 144) and EBSCOhost (n = 11). After 50 duplicated articles were removed, the 153 articles were excluded by screening the title and abstract: 59 articles are reviews or books; 11 articles do not provide the clinical data; 16 articles are related to the other genes; 46 articles focus on non-NAFLD diseases; 21 articles are case, trial or fail to contain the data of
gene polymorphism. 18 potentially articles were then assessed for eligibility. The data was extracted from all these full-text articles. As shown in S2 Text, 6 articles were meeting/conference abstracts and 4 articles were lack of usable data. We failed to obtain missing data. Finally, 8 articles (785 cases and 1188 controls) fulfilled the inclusion criteria and were included in the present meta-analysis [23–30]. The data was extracted independently by the authors (MYS LZ SLS JNL). The characteristics of included articles were summarized and showed in Table 1. All the case-control studies were population-based. This meta-analysis was carried out according to the recommendations of the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA) statement (S1 Table) and "Meta-analysis on Genetic Association Studies" statement (S2 Table) [37].

Table 1. Characteristics of eligible studies in meta-analysis.

| first author      | year | country | ethnicity | sample sizes | source of control | genotyping method                        | case gender (male %) | age (year) |
|-------------------|------|---------|-----------|--------------|-------------------|-----------------------------------------|----------------------|------------|
| Chen et al.       | 2014 | China   | Asian     | 212          | PB                | PCR-gene CHIP                          | 60.8                 | 40–54      |
| de Carvalho et al.| 2013 | Brazil  | Caucasian | 35           | PB                | PCR-RFLP/PCR-ASA                       | 25.7                 | mean 49    |
| Franco et al.     | 2013 | Brazil  | Caucasian | 134          | PB                | PCR-RFLP                               | 42.5                 | 32–56      |
| Hu et al.         | 2009 | China   | Asian     | 63           | PB                | PCR-RFLP                               | NA                   | NA         |
| Kasapoglu et al.  | 2015 | Turkey  | Caucasian | 150          | PB                | PCR-SSCP                               | 30.0                 | 32–63      |
| Orlovskaia et al. | 2015 | Ukraine | Caucasian | 100          | PB                | PCR-fluorescence hybridization          | NA                   | NA         |
| Sazci et al.      | 2008 | Turkey  | Caucasian | 57           | PB                | PCR-RFLP                               | 54.4                 | 18–66      |
| Serin et al.      | 2007 | Turkey  | Caucasian | 34           | PB                | PCR-RFLP                               | 55.9                 | 33–51      |

PB: population-based; NA: not available; PCR-RFLP: polymerase chain reaction–restriction fragment length polymorphism; PCR-ASA: polymerase chain reaction–amplicon sequence analysis; PCR-SSCP: Polymerase chain reaction-single strand conformation polymorphism.
Meta-analysis on the association between NAFLD risks and C677T polymorphism of MTHFR

Next, the genetic association between MTHFR C677T polymorphism and susceptibility to NAFLD was measured. As shown in Fig 2A, the result ($I^2 = 56\%$ and $P = 0.004$) revealed that high heterogeneity among studies was detected for C677T polymorphism. Random-effect model was thus applied for meta-analysis. The data on the association between C677T allele frequency of MTHFR and the risks of NAFLD.

### Table 1: Meta-analysis Results for C677T Polymorphism

| Study or Subgroup | Decreased risk | Increased risk | Weight | M-H, Random, 95% CI | Odds Ratio M-H, Random, 95% CI |
|-------------------|---------------|---------------|--------|---------------------|-------------------------------|
| Chen 2014         | 181           | 424           | 121    | 350                 | 1.41 [1.05, 1.89]             |
| de Carvalho 2013  | 29            | 70            | 24     | 90                  | 1.95 [1.00, 3.79]             |
| Franco 2013       | 97            | 268           | 101    | 268                 | 0.94 [0.66, 1.33]             |
| Hu 2009           | 64            | 126           | 35     | 104                 | 2.04 [1.19, 3.48]             |
| Kasapoglu 2015a   | 66            | 124           | 80     | 216                 | 1.93 [1.24, 3.03]             |
| Kasapoglu 2015b   | 28            | 48            | 80     | 216                 | 2.38 [1.26, 4.50]             |
| Kasapoglu 2015c   | 18            | 32            | 80     | 216                 | 2.19 [1.03, 4.63]             |
| Orlovskiy 2015a   | 70            | 200           | 28     | 80                  | 1.12 [0.64, 1.94]             |
| Orlovskiy 2015b   | 33            | 106           | 26     | 80                  | 0.94 [0.50, 1.75]             |
| Orlovskiy 2015c   | 37            | 94            | 26     | 80                  | 1.35 [0.72, 2.52]             |
| Sazci 2008a       | 29            | 114           | 187    | 648                 | 0.84 [0.53, 1.32]             |
| Sazci 2008b       | 14            | 62            | 105    | 342                 | 0.66 [0.35, 1.25]             |
| Sazci 2008c       | 15            | 52            | 82     | 306                 | 1.11 [0.58, 2.12]             |
| Serin 2007a       | 29            | 68            | 277    | 564                 | 0.77 [0.46, 1.28]             |
| Serin 2007b       | 8             | 24            | 277    | 564                 | 0.52 [0.22, 1.23]             |
| Serin 2007c       | 21            | 44            | 277    | 564                 | 0.95 [0.51, 1.75]             |
| Total (95% CI)    | 1856          | 4688          | 100.0% | 1.20 [0.98, 1.47]   |

Total events: 739

Heterogeneity: Tau² = 0.09; Chi² = 33.77, df = 15 (P = 0.004); I² = 56%

Test for overall effect: Z = 1.80 (P = 0.07)

### Figure 2: Meta-analysis Results

(A) Forest plot under T vs C model; (B) Begg’s funnel plot of publication biases under T vs C model.

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The frequency of MTHFR and susceptibility to NAFLD was obtained (T vs C, OR = 1.20, 95% CI = 0.98−1.47, \(P = 0.07\)). In addition, the potential publication bias was evaluated by Begg’s funnel plot with pseudo 95% confidence limits. The result of Fig 2B suggested that basically symmetric plot (\(z = 0.14, P = 0.893\)) excludes the presence of large publication bias.

The contrast of the homozygote model (T/T vs C/C), heterozygote model (C/T vs C/C), dominant model (C/T+T/T vs C/C) and recessive model (T/T vs C/C+C/T) was then detected respectively, through the meta-analysis, in that the data on genotype frequencies of MTHFR C677T polymorphism was available. Genotype distribution and characteristics of MTHFR C677T polymorphism in different case-control studies were shown in Table 2. The T/T vs C/C (\(I^2 = 47\%\) and \(P = 0.02\), C/T+T/T vs C/C (\(I^2 = 45\%\) and \(P = 0.03\)) and T/T vs C/C+C/T (\(I^2 = 26\%\) and \(P = 0.16\)) data indicated the existence of the moderate degree of heterogeneity across studies (Table 3). A random-effect model was thus used. However, fixed-effect model was used for the C/T vs C/C model (\(I^2 = 21\%\) and \(P = 0.21\)). Pooled analysis for the association between C677T genotype frequencies and the risks of NAFLD was shown in Table 3. Briefly, compared with control group, an increased NAFLD risk was observed in case group under homozygote
Table 3. Pooled analysis for the association between MTHFR C677T genotype frequencies and the risks of NAFLD.

| Comparison                  | study          | Events | Total | Events | Total | OR (95% CI) | P value | I² (%) | P value |
|-----------------------------|----------------|--------|-------|--------|-------|-------------|---------|--------|---------|
| T/T vs C/C (homozygote)    | de Carvalho,2013 | 6      | 18    | 2      | 25    | 5.75 [1.00, 32.95] | 0.04    | 47     | 0.02    | R       |
|                             | Franco,2013     | 20     | 77    | 17     | 67    | 1.03 [0.49, 2.18]  |         |        |         |         |
|                             | Chen,2014       | 40     | 111   | 28     | 110   | 1.65 [0.93, 2.94]  |         |        |         |         |
|                             | Kasapoglu,2015  | 16     | 28    | 12     | 52    | 4.44 [1.65, 11.94] |         |        |         |         |
|                             |                | 8      | 12    | 12     | 52    | 6.67 [1.71, 26.04] |         |        |         |         |
|                             | Hu,2009         | 22     | 43    | 9      | 35    | 3.03 [1.15, 7.95]  |         |        |         |         |
|                             | Sazci,2008      | 4      | 36    | 24     | 185   | 0.84 [0.27, 2.58]  |         |        |         |         |
|                             |                | 2      | 21    | 15     | 96    | 0.57 [0.12, 2.70]  |         |        |         |         |
|                             |                | 2      | 15    | 9      | 89    | 1.37 [0.27, 7.05]  |         |        |         |         |
|                             | Serin,2007      | 14     | 33    | 126    | 257   | 0.77 [0.37, 1.59]  |         |        |         |         |
|                             |                | 4      | 12    | 126    | 257   | 0.52 [0.15, 1.77]  |         |        |         |         |
|                             |                | 10     | 21    | 126    | 257   | 0.95 [0.39, 2.30]  |         |        |         |         |
| C/T vs C/C (heterozygote)   | de Carvalho,2013 | 17     | 29    | 20     | 43    | 1.63 [0.63, 4.22]  |         |        |         |         |
|                             | Franco,2013     | 57     | 114   | 67     | 117   | 0.75 [0.44, 1.25]  |         |        |         |         |
|                             | Chen,2014       | 101    | 172   | 65     | 147   | 1.79 [1.15, 2.80]  |         |        |         |         |
|                             | Kasapoglu,2015  | 34     | 46    | 56     | 96    | 2.02 [0.93, 4.38]  |         |        |         |         |
|                             |                | 12     | 16    | 56     | 96    | 2.14 [0.64, 7.13]  |         |        |         |         |
|                             | Hu,2009         | 20     | 41    | 17     | 43    | 1.46 [0.61, 3.46]  |         |        |         |         |
|                             | Sazci,2008      | 21     | 53    | 139    | 300   | 0.76 [0.42, 1.38]  |         |        |         |         |
|                             |                | 10     | 29    | 75     | 156   | 0.57 [0.25, 1.30]  |         |        |         |         |
|                             |                | 11     | 24    | 64     | 144   | 1.06 [0.44, 2.52]  |         |        |         |         |
|                             | Serin,2007      | 1      | 20    | 25     | 156   | 0.28 [0.04, 1.15]  |         |        |         |         |
|                             |                | 0      | 8     | 25     | 156   | 0.30 [0.02, 5.42]  |         |        |         |         |
|                             |                | 1      | 12    | 25     | 156   | 0.48 [0.06, 3.86]  |         |        |         |         |
| C/T+T/T vs C/C (dominant)   | de Carvalho,2013 | 23     | 35    | 22     | 45    | 2.00 [0.81, 4.98]  |         |        |         |         |
|                             | Franco,2013     | 77     | 134   | 84     | 134   | 0.80 [0.49, 1.31]  |         |        |         |         |
|                             | Chen,2014       | 141    | 212   | 93     | 175   | 1.75 [1.16, 2.64]  |         |        |         |         |
|                             | Kasapoglu,2015  | 50     | 62    | 68     | 108   | 2.45 [1.17, 5.14]  |         |        |         |         |
|                             |                | 20     | 24    | 68     | 108   | 2.94 [0.94, 9.22]  |         |        |         |         |
|                             | Hu,2009         | 42     | 63    | 26     | 52    | 2.00 [0.94, 4.25]  |         |        |         |         |
|                             | Sazci,2008      | 25     | 57    | 163    | 324   | 0.77 [0.44, 1.36]  |         |        |         |         |

(Continued)
model (T/T vs C/C, OR = 1.49, 95% CI = 1.03–2.15, P = 0.04) and recessive model (T/T vs C/ C+C/T, OR = 1.42, 95% CI = 1.07–1.88, P = 0.02), but not the other genetics models (C/T vs C/ C, OR = 1.14, 95% CI = 0.93–1.39, P = 0.21; C/T+T/T vs C/C, OR = 1.18, 95% CI = 0.91–1.52, P = 0.21). In addition, the results of HWE test (Table 2) in control group of two studies [24, 25] indicated that the genotype distributions deviated from HWE (Χ² = 5.605, P = 0.018; Χ² = 190.839, P < 0.05). The subgroup analyses under all genetic models were also performed based on ethnicity or HWE (Table 4) via Stata/SE 12.0 software. A significantly increased NAFLD risk was observed in Asian population (T vs C, OR = 1.58, 95% CI = 1.13–2.20, P = 0.007; T/T vs C/C, OR = 1.97, 95% CI = 1.15–3.37, P = 0.014; C/T vs C/C, OR = 1.72, 95% CI = 1.16–2.55, P = 0.007; C/T+T/T vs C/C, OR = 1.81, 95% CI = 1.26–2.59, P = 0.001) and HWE P > 0.05 subgroup (T vs C, OR = 1.31, 95% CI = 1.03–1.67, P = 0.030; T/T vs C/C, OR = 1.85, 95% CI = 1.15–2.97, P = 0.011; T/T vs C+C/T, OR = 1.72, 95% CI = 1.21–2.46, P = 0.003). In order to evaluate the influence of each study on the overall OR under all genetic models, the sensitivity meta-analyses, in which one study is omitted at a time, were also performed. As shown in Fig 3, the results indicated that the corresponding pooled OR value did not differ significantly from that of the overall meta-analysis. Furthermore, no significant publication bias was observed in all above genetic models via Begg’s funnel plot and Egger’s test (Data not shown), suggesting these results are reliable. These data indicated that the T/T genotype of MTHFR C677T polymorphism seems to be associated with genetic susceptibility to NAFLD, especially in Asian population.
Meta-analysis on the association between NAFLD risks and A1298C polymorphism of MTHFR

Besides C677T, meta-analysis on the association between MTHFR A1298C polymorphism and NAFLD risks was also performed. Table 2 showed the genotype distribution and characteristics of MTHFR A1298C polymorphism. All the control groups of these studies were in line with HWE (All \( P > 0.05 \)). In addition, all the case-control studies were performed in Caucasian population. We then first performed the meta-analysis between the allele frequency of MTHFR A1298C and the susceptibility to NAFLD under C vs A model. As shown in Fig 4A, random-effect model was used, due to the existence of high between-studies heterogeneity (I\(^2\) = 66\% and \( P = 0.001 \)) for meta-analysis. The data (OR = 1.53, 95\% CI = 1.13–2.07, \( P = 0.006 \)) was
obtained in C vs A comparison of MTHFR A1298C. The basically symmetric plot ($z = 0.93$, $P = 0.350$) did not provide the statistical evidence for publication bias (Fig 4B).

Moreover, we also performed the pooled analysis for the associations between MTHFR genotype frequencies of A1298C and the susceptibility to NAFLD (Table 5). The data of C/C vs A/A model ($I^2 = 39\%$ and $P = 0.09$), A/C vs A/A model ($I^2 = 53\%$ and $P = 0.02$), A/C+C/C vs A/A model ($I^2 = 63\%$ and $P = 0.003$) was obtained and random-effect model was used. For the C/C vs A/A+A/C model, fixed-effect model was used ($I^2 = 14\%$ and $P = 0.31$). A significantly increased NAFLD risks was observed in homozygote model (C/C vs A/A, OR = 2.81, 95% CI = 1.63–4.85, $P = 0.0002$), dominant model (A/C+C/C vs A/A, OR = 1.60, 95% CI = 1.06–2.41, $P = 0.03$) and recessive models (C/C vs A/A+A/C, OR = 2.08, 95% CI = 1.45–3.00, $P < 0.0001$), but not heterozygote model (A/C vs A/A, OR = 1.38, 95% CI = 0.94–2.03, $P = 0.10$). Moreover, similar results were obtained in the sensitivity meta-analyses under all genetic models (Fig 5). These data suggested that C/C genotype of
MTHFR A1298C polymorphism is more likely to be strongly associated with the susceptibility to NAFLD in Caucasian population.

**Discussion**

Several studies have reported the potential association between the most common allelic variants of MTHFR gene (C677T and A1298C) and susceptibility to many clinical diseases, such as gastric cancer, hepatocellular carcinoma, NAFLD, neural tube defects, acute lymphoblastic leukemia and renal/heart failure [11, 25, 26, 38–42]. For example, MTHFR C677T polymorphism is found to be linked to an increased risk of neural tube defects [40]; MTHFR gene mutations...
| Comparison                  | study                | case | control | Test of association | Heterogeneity | Model |
|-----------------------------|----------------------|------|---------|---------------------|---------------|-------|
|                             |                      | Events | Total Events | Total OR (95% CI) | $P$ value | $I^2$ (%) | $P$ value |
| C/C vs A/A (homozygote)     |                      | C/C   | C/C     | 2.81 [1.63, 4.85]  | 0.0002 | 39       | 0.09      |
| de Carvalho,2013            | 0                    | 20    | 2       | 0.26 [0.01, 5.69]  |              |         |           |
| Franco,2013                 | 7                    | 81    | 6       | 1.02 [0.33, 3.20]  |              |         |           |
| Kasapoglu,2015              | 8                    | 20    | 4       | 6.67 [1.71, 26.04] |              |         |           |
|                             | 6                    | 10    | 4       | 15.00 [2.94, 76.56] |              |         |           |
|                             | 3                    | 6     | 4       | 10.00 [1.49, 66.99] |              |         |           |
| Orlovsky,2015               | 13                   | 66    | 3       | 1.64 [0.42, 6.35]  |              |         |           |
|                             | 4                    | 36    | 3       | 0.83 [0.17, 4.12]  |              |         |           |
|                             | 9                    | 31    | 3       | 2.73 [0.65, 11.51] |              |         |           |
| Sazci,2008                  | 10                   | 23    | 33      | 3.19 [1.29, 7.92]  |              |         |           |
|                             | 4                    | 11    | 19      | 2.20 [0.58, 8.29]  |              |         |           |
|                             | 6                    | 12    | 14      | 4.57 [1.28, 16.29] |              |         |           |
| A/C vs A/A (heterozygote)   |                      | A/C   | A/C     | 1.38 [0.94, 2.03]  | 0.10 | 53       | 0.02      |
| de Carvalho,2013            | 15                   | 35    | 17      | 1.15 [0.46, 2.84]  |              |         |           |
| Franco,2013                 | 53                   | 127   | 63      | 0.74 [0.45, 1.21]  |              |         |           |
| Kasapoglu,2015              | 18                   | 30    | 24      | 2.50 [1.03, 6.08]  |              |         |           |
|                             | 8                    | 12    | 24      | 3.33 [0.91, 12.26] |              |         |           |
|                             | 5                    | 8     | 24      | 2.78 [0.61, 12.68] |              |         |           |
| Orlovsky,2015               | 34                   | 87    | 17      | 0.75 [0.35, 1.64]  |              |         |           |
|                             | 17                   | 49    | 17      | 0.63 [0.26, 1.50]  |              |         |           |
|                             | 16                   | 38    | 17      | 0.86 [0.34, 2.13]  |              |         |           |
| Sazci,2008                  | 34                   | 154   | 291     | 2.33 [1.18, 4.59]  |              |         |           |
|                             | 20                   | 27    | 79      | 2.64 [1.05, 6.61]  |              |         |           |
|                             | 14                   | 20    | 75      | 1.99 [0.72, 5.48]  |              |         |           |
| A/C+C/C vs A/A (dominant)   |                      | A/C+C/C | A/C+C/C | 1.60 [1.06, 2.41]  | 0.03 | 63       | 0.003     |
| de Carvalho,2013            | 15                   | 35    | 19      | 1.03 [0.42, 2.51]  |              |         |           |
| Franco,2013                 | 60                   | 134   | 69      | 0.76 [0.47, 1.23]  |              |         |           |
| Kasapoglu,2015              | 26                   | 38    | 28      | 3.10 [1.34, 7.15]  |              |         |           |
|                             | 14                   | 18    | 28      | 5.00 [1.49, 16.79] |              |         |           |
|                             | 8                    | 11    | 28      | 3.81 [0.93, 15.64] |              |         |           |
| Orlovsky,2015               | 47                   | 100   | 20      | 0.89 [0.43, 1.85]  |              |         |           |
|                             | 21                   | 53    | 20      | 0.66 [0.29, 1.50]  |              |         |           |
|                             | 25                   | 47    | 20      | 1.14 [0.49, 2.64]  |              |         |           |
| Sazci,2008                  | 44                   | 187   | 324     | 2.48 [1.29, 4.78]  |              |         |           |
|                             | 24                   | 31    | 98      | 2.55 [1.04, 6.25]  |              |         |           |
|                             | 20                   | 26    | 89      | 2.40 [0.91, 6.31]  |              |         |           |
| C/C vs A/A+A/C (recessive)  |                      | C/C   | C/C     | 2.08 [1.45, 3.00]  | <0.0001 | 14       | 0.31      |
| de Carvalho,2013            | 0                    | 35    | 2       | 0.25 [0.01, 5.27]  |              |         |           |
| Franco,2013                 | 7                    | 134   | 6       | 1.18 [0.38, 3.60]  |              |         |           |
| Kasapoglu,2015              | 8                    | 38    | 4       | 4.27 [1.19, 15.29] |              |         |           |
|                             | 6                    | 18    | 4       | 8.00 [1.96, 32.68] |              |         |           |
|                             | 3                    | 11    | 4       | 6.00 [1.13, 31.80] |              |         |           |
| Orlovsky,2015               | 13                   | 100   | 3       | 1.84 [0.50, 6.85]  |              |         |           |
|                             | 4                    | 53    | 3       | 1.01 [0.21, 4.78]  |              |         |           |
|                             | 9                    | 47    | 3       | 2.92 [0.73, 11.64] |              |         |           |
| Sazci,2008                  | 10                   | 57    | 33      | 1.88 [0.87, 4.06]  |              |         |           |

(Continued)
might be conductive to renal function in Italian population [42]. However, the effect of MTHFR polymorphisms in the presence of NAFLD remains inconclusive in different populations [22–30]. For instance, C677T and A1298C polymorphisms of MTHFR gene were significantly associated with NASH risks in Turkish population [26]. The association of MTHFR A1298C polymorphism with NAFLD severity was also observed in Italy population [22].

Table 5. (Continued)

| Comparison | study | case Events | Total | control Events | Total | Test of association OR (95% CI) | P value | Heterogeneity I² (%) | Model P value |
|------------|-------|-------------|-------|----------------|-------|-------------------------------|---------|---------------------|-------------|
|            |       | 4           | 31    | 19             | 171   | 1.19 [0.37, 3.76]             |         |                     |             |
|            |       | 6           | 26    | 14             | 153   | 2.98 [1.03, 8.64]             |         |                     |             |

R: Random-effect; F: Fixed-effect.

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Fig 5. The sensitivity meta-analyses for the association between A1298C polymorphism of MTHFR and the risks of NAFLD.

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However, both MTHFR C677T and A1298C polymorphisms were not considered as the potential genetic risk factors for the development of NAFLD in Brazilian population [29]. The data of Serin et al also suggested that MTHFR C677T polymorphism is unlikely to be associated with the progression of non-alcoholic fatty liver to NASH in their Turkish cohort study [25]. Here, a meta-analysis was first conducted to further comprehensively evaluate the genetic association, based on the data from all available population-based case-control studies.

The positive correlation between NAFLD susceptibility and two MTHFR variants (C677T and A1298C) was observed in our statistical evidence. For C677T polymorphism, an increased NAFLD risk was observed under homozygote model (T/T vs C/C) and recessive model (T/T vs C/C+C/T), but not T vs C, C/T vs C/C and C/T+T/T vs C/C models, suggesting that T/T genotype of MTHFR C677T polymorphism might have the increased risks of NAFLD in general population. Moreover, we found that a significantly increased NAFLD risk was detected in Asian population under the comparison of T vs C, T/T vs C/C, C/T vs C/C; C/T+T/T vs C/C. Similarly, the meta-analysis of A1298C polymorphism based on 11 case-control studies in Caucasian population provided the evidence that a significantly increased NAFLD risk was observed under allele model (C vs A), homozygote model (C/C vs A/A), dominant model (A/C + C/C vs A/C) and recessive model (C/C vs A/A+A/C), but not heterozygote model (A/C vs A/A), suggesting that C/C genotype of MTHFR A1298C polymorphism might be linked to the susceptibility to NAFLD in Caucasian population.

The C677T polymorphism means the substitution of C (cytosine) to T (thymine) at nucleotide position 677, which results in the transition from alanine to valine, while A1298C polymorphism refers to the transition of A (adenine) to C (cytosine) at position 1298, which leads to an amino acid substitution from glutamic acid to alanine [8–11]. Folate is closely associated with the synthesis, methylation and repair of DNA, and is essential for the production or maintenance of normal cell and the inhibition of tumor cells [43–45]. The mutations of MTHFR gene were reported to reduce the enzyme activity of MTHFR, concentration of folate, and thus take part in the up-regulation of serum Hcy levels [6, 46, 47]. Kasapoglu B, et al. reported that homozygote mutations of MTHFR C677T and A1298C are positively associated with the increased levels of serum Hcy in NAFLD individuals [28]. Here, individuals, who carry T/T genotype in C677T and C/C genotype in A1298C polymorphism, might have high risks of NAFLD. It is possible that the two harmful homozygous mutations of MTHFR gene confer susceptibility to NAFLD via the abnormality of MTHFR enzyme activity and folate-involved DNA metabolism. Intriguingly, homozygote C/C genotype of MTHFR A1298C seems to be significantly linked to a decreased risk of liver cancer in Asian population, whereas homozygote T/T genotype of MTHFR C677T shows a reversed effect [38, 48, 49]. More experiments are needed to investigate the molecular mechanism on the distinct roles of MTHFR polymorphisms in the occurrence of NAFLD and hepatic carcinoma.

There are some shortages or limitations in this meta-analysis, which should be pointed out. For example, no large sample size was included in the case/control groups of meta-analysis. It is still possible that other unpublished or undetected studies are present, although we selected the eligible studies independently according to the inclusion and exclusion criteria. The potential selection bias still may affect the reliability of our findings. Different degree of heterogeneity and departure from HWE was also detected in some comparisons or case-control studies. Furthermore, it was reported that C677C/C1298C compound genotype confers increased risks of NASH in Turkish women patients [26]. Unfortunately, we failed to carry out the meta-analysis to investigate the potential role of MTHFR susceptibility loci combination in the susceptibility to NAFLD, due to the limitation of relevant data.

Very complicated natural history of NAFLD was existed, and multiple genetic or environmental factors contribute to the occurrence and progression of the NAFLD [50–53]. NAFLD
has become a public health concern for its close relation with the other metabolic syndrome, hyperhomocysteinemia, obesity, hypertension, type 2 diabetes mellitus, cardiovascular disease or hepatocellular carcinoma [50–54]. Accumulating evidence showed the relationship between the MTHFR polymorphism and the pathogenesis of NAFLD-associated diseases [38, 42, 55–57]. To perform more frequent screening of functional MTHFR gene variants and other potential clinical characteristics is useful to reduce the development of the above diseases. Larger and well-designed studies and further meta-analyses based on population feature, disease status, gender, geographical location, detailed information of diet or physical activity are required to study the role of MTHFR mutation in the risks of NAFLD and NAFLD-associated diseases.

Conclusion

All in all, this is the first meta-analysis to provide evidence that C677T and A1298C mutations of MTHFR are significantly associated with an increased risk of NAFLD. The homozygous T/T genotype of MTHFR C677T and C/C genotype of MTHFR A1298C polymorphism seem to be more susceptible to NAFLD. More case-control studies are warranted to validate the conclusion.

Supporting Information

S1 Table. PRISMA 2009 checklist. (DOCX)
S2 Table. meta-analysis on genetic association studies form. (DOCX)
S1 Text. The full details of databases searching terms. (DOCX)
S2 Text. Full-text articles excluded with reasons. (DOCX)

Author Contributions

Conceived and designed the experiments: MYS JNL. Performed the experiments: MYS LZ SLS JNL. Analyzed the data: MYS LZ SLS JNL. Contributed reagents/materials/analysis tools: JNL. Wrote the paper: MYS.

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