MTHFR 677C>T Polymorphism Increases the Male Infertility Risk: A Meta-Analysis Involving 26 Studies

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Abstract

Background and Objectives
Methylenetetrahydrofolate reductase (MTHFR) polymorphism may be a risk factor for male infertility. However, the epidemiologic studies showed inconsistent results regarding MTHFR polymorphism and the risk of male infertility. Therefore, we performed a meta-analysis of published case-control studies to re-examine the controversy.

Methods
Electronic searches of PubMed, EMBASE, Google Scholar and China National Knowledge Infrastructure (CNKI) were conducted to select eligible literatures for this meta-analysis (updated to June 19, 2014). According to our inclusion criteria and the Newcastle-Ottawa Scale (NOS), only high quality studies that observed the association between MTHFR polymorphism and male infertility risk were included. Crude odds ratio (OR) with 95% confidence interval (CI) was used to assess the strength of association between the MTHFR polymorphism and male infertility risk.

Results
Twenty-six studies involving 5,575 cases and 5,447 controls were recruited. Overall, MTHFR 677C>T polymorphism showed significant associations with male infertility risk in both fixed effects (CT+TT vs. CC: OR = 1.34, 95% CI: 1.23–1.46) and random effects models (CT+TT vs. CC: OR = 1.39, 95% CI: 1.19–1.62). Further, when stratified by ethnicity, sperm concentration and control sources, the similar results were observed in Asians,
Caucasians, Azoo or OAT subgroup and both in population-based and hospital-based controls. Nevertheless, no significant association was only observed in oligo subgroup.

Conclusions

Our results indicated that the MTHFR polymorphism is associated with an increased risk of male infertility. Further well-designed analytical studies are necessary to confirm our conclusions and evaluate gene-environment interactions with male infertility risk.

Introduction

Infertility has been acknowledged as a very common health problem that affects approximately 15%-20% of couples who want to conceive [1], and almost 50% cases are because of male factors. Despite significant advancements in the male infertility diagnoses, the etiology remains unknown in almost half of all male infertile cases [2]. However, spermatogenic failure is the most common phenomenon among these cases. At present, it has been postulated that genetic abnormalities are thought to account for 15%-30% of male factor infertility, which include Y chromosome microdeletions, translocation, chromosomal aberrations and single-gene mutations [3–6]. In many infertile men, deleterious gene polymorphisms in key genes involved in testicular function, combined with environmental factors, may be responsible for the poor quality and number reduction of the sperm.

Folate is very important for the maintenance of genome integrity due to its role in DNA synthesis, repair and methylation [7, 8]. It is known that folate deficiency occur frequently, and the related hyperhomocysteinaemia is considered as a risk factor for various diseases, including infertility. Methylenetetrahydrofolate reductase (MTHFR) is one of the key regulatory enzymes in folate metabolism which can catalyze the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the methyl donor for homocysteine in the synthesis of methionine. Subsequently, methionine in its activated form S-adenosylmethionine is the methyl donor for DNA methylation [9]. Methylation anomalies of sperm DNA has been linked to male infertility [10]. Moreover, it has been reported that the folate metabolic pathways can be modified by polymorphisms of relevant genes such as MTHFR or by the action of carcinogenic elements, for example, alcohol or tobacco [11].

The MTHFR gene, located on the short arm of chromosome1 (1p36.3), which is composed of 11 exons [12, 13]. The change of C for T at the nucleotide position 677 of the MTHFR gene causes the substitution of valine for alanine in the MTHFR protein and a consequent reduction in enzyme activity. The MTHFR 677C>T variant decreases the activity of the enzyme by 35% in the presence of heterozygosis and by 70% in homozygosis [12]. Reduced enzymatic activity due to MTHFR polymorphisms is considered as a risk factor for many diseases, including infertility [14].

Recent years, a number of epidemiological studies have been conducted to examine the association between MTHFR 677C>T polymorphism and male infertility risk in diverse populations, but the results of these studies remain conflicting rather than conclusive. Some studies exhibited significantly increased risk of male infertility with MTHFR 677C>T, while some other studies showed nonsignificantly enhanced risk. As a result, there were five meta-analyses [15–19] performed to examine the association between MTHFR 677C>T polymorphism and the risk of male infertility, however, the results still inconsistent. Moreover, many new
researches studied the association between male infertility risk and MTHFR 677C>T after the last meta-analysis researching, so an updated and high quality meta-analysis is needed.

In order to evaluate the association between the MTHFR 677C>T polymorphism and male infertility risk, we carried out a meta-analysis with subgroup analysis using all the eligible published data until June 19, 2014.

Materials and Methods

Search Strategy and Selection Criteria

According to the Meta-analysis on Genetic Association Studies Checklist (S1 Checklist), we conducted a computer-based systematic search of PubMed, EMBASE, Google Scholar and China National Knowledge Infrastructure (CNKI) without restriction on language (updated to June 19, 2014). The key words were as follows: “methylene tetrahydrofolate reductase” or “MTHFR”, “polymorphism” or “variant”, “infertility” or “azoospermia” or “oligoasthenoteratozoospermia” or “oligozoospermia” or “subfertility”. In addition, we checked the references of all eligible articles which our research retrieved. For the meta-analysis, the following inclusion criteria were considered: (1) studied on human beings; (2) studies which evaluated the association between MTHFR 677C>T polymorphism and male infertility risk; (3) studies with case-control design; (4) sufficient published data about the size of the sample, odds ratio (OR), and their 95% confidence interval (CI). For the exclusion criteria, we provided as follows: (1) without raw data for the calculation of odds ratios (ORs) with corresponding 95% confidence intervals (95% CIs); (2) when studies with overlapping cases or controls, we included only the most recent or the largest report.

Data Extraction

According to the inclusion and exclusion criteria, the two investigators (Tingyu He and Zhirong Shi) extracted raw data independently in order to ensure the accuracy of extracted information. For conflicting evaluations, an agreement was reached following a discussion. The following information was collected from all eligible studies showing in Table 1: the surname of the first author, date of publication, quality scores, ethnicity, sperm concentration subgroup categories, sources of controls, number of cases and controls and the P value of Hardy Weinberg Equilibrium (HWE). Different ethnic groups were mainly categorized as Caucasian, Asian and African. According to the sperm concentration, we divided the subgroup as azoospermia (Azoo), oligoasthenoteratozoospermia (OAT) and oligozoospermia (oligo) groups. Study designs were stratified to population-based studies and hospital-based studies.

Quality assessment

Three authors (Guiying Huang, Rui Ren and Sichong Huang) assessed the study quality independently based on the Newcastle-Ottawa Scale [20], which uses a star rating system to judge the methodological quality. A full score is 9 stars, and a score range 5 to 9 stars is considered to be a generally high methodological quality while a score range 0 to 4 is considered to be a poor quality [21]. The quality of all included studies was summarized in Table 2. Any disagreements on the NOS score of the studies were resolved through a comprehensive reassessment by the other authors and only high quality studies can be included in our meta-analysis.

Statistical Analysis

The association between MTHFR 677C>T polymorphism and the male infertility risk were estimated by pooled ORs with 95% CI. The statistical significance of the pooled ORs was
Table 1. Characteristics of eligible studies in the meta-analysis of MTHFR 677C>T polymorphism and male infertility.

| First author | Year | Quality scores | Group | Design | Cases Total | CC | CT | TT | Controls Total | CC | CT | TT | P HWE |
|--------------|------|----------------|-------|--------|-------------|----|----|----|----------------|----|----|----|-------|
| **Asians**   |      |                |       |        |             |    |    |    |                 |    |    |    |       |
| Park         | 2005 | 6              | Total | HB     | 373         | 105| 205| 63 | 396            | 145| 200| 51 | 0.161 |
|              |      |                | Azoo  |        | 286         | 75 | 164| 47 |                 |    |    |    |       |
|              |      |                | OAT   |        | 85          | 28 | 40 | 17 |                 |    |    |    |       |
| Lee          | 2006 | 6              | Total | HB     | 360         | 115| 181| 64 | 325            | 118| 166| 41 | 0.138 |
|              |      |                | Azoo  |        | 174         | 44 | 100| 30 |                 |    |    |    |       |
|              |      |                | OAT   |        | 186         | 71 | 81 | 34 |                 |    |    |    |       |
| A            | 2007 | 6              | Total | NA     | 355         | 130| 160| 65 | 252            | 128| 95 | 29 | 0.085 |
|              |      |                | Azoo  |        | 228         | 83 | 97 | 48 |                 |    |    |    |       |
|              |      |                | oligo |       | 127         | 47 | 63 | 17 |                 |    |    |    |       |
| Sun          | 2007 | 5              | Total | PB     | 182         | 27 | 86 | 69 | 53             | 15 | 28 | 10 | 0.630 |
|              |      |                | OAT   |        | 22          | 3  | 11 | 8  |                 |    |    |    |       |
|              |      |                | oligo |       | 46          | 10 | 20 | 16 |                 |    |    |    |       |
| Yang         | 2010 | 5              | Total | PB     | 131         | 34 | 55 | 42 | 293            | 98 | 142| 53 | 0.901 |
| Qiu          | 2011 | 6              | Total | NA     | 271         | 75 | 112| 84 | 180            | 63 | 85 | 32 | 0.720 |
|              |      |                | Azoo  |        | 158         | 42 | 66 | 50 |                 |    |    |    |       |
|              |      |                | oligo |       | 113         | 33 | 46 | 34 |                 |    |    |    |       |
| Safarinejad  | 2011 | 6              | OAT   | HB     | 164         | 58 | 80 | 26 | 328            | 144| 148| 36 | 0.825 |
| Liu          | 2012 | 7              | Total | PB     | 75          | 27 | 38 | 10 | 72             | 40 | 28 | 4  | 0.753 |
| Pei          | 2013 | 7              | Total | PB     | 290         | 39 | 138| 113| 90             | 24 | 47 | 19 | 0.651 |
| Li           | 2014 | 6              | Total | HB     | 82          | 14 | 36 | 32 | 133            | 36 | 61 | 36 | 0.340 |
| **Caucasians** |      |                |       |        |             |    |    |    |                 |    |    |    |       |
| Ebisch       | 2003 | 7              | Total | PB     | 77          | 42 | 28 | 7  | 113            | 50 | 48 | 15 | 0.522 |
| Stuppia      | 2003 | 5              | Total | NA     | 93          | 37 | 37 | 19 | 105            | 33 | 43 | 29 | 0.065 |
|              |      |                | Azoo  |        | 21          | 8  | 6  | 7  |                 |    |    |    |       |
|              |      |                | oligo |       | 66          | 25 | 29 | 12 |                 |    |    |    |       |
| Singh        | 2005 | 6              | Total | PB     | 151         | 105| 40 | 6  | 200            | 163| 37 | 0  | 0.149 |
| Paracchini   | 2006 | 6              | Total | PB     | 59          | 11 | 32 | 16 | 46             | 18 | 21 | 7  | 0.830 |
| Ravel        | 2009 | 6              | Total | HB     | 250         | 118| 101| 31 | 114            | 49 | 52 | 13 | 0.887 |
|              |      |                | Azoo  |        | 70          | 33 | 31 | 6  |                 |    |    |    |       |
|              |      |                | oligo |       | 180         | 85 | 70 | 25 |                 |    |    |    |       |
| Murphy       | 2011 | 7              | Total | PB     | 149         | 73 | 63 | 13 | 182            | 94 | 73 | 15 | 0.876 |
| Gupta        | 2011 | 5              | Total | HB     | 522         | 378| 116| 28 | 315            | 251| 58 | 6  | 0.228 |
|              |      |                | Azoo  |        | 68          | 49 | 15 | 4  |                 |    |    |    |       |
|              |      |                | OAT   |        | 65          | 41 | 23 | 1  |                 |    |    |    |       |
| Chellat      | 2012 | 5              | Total | NA     | 74          | 31 | 33 | 10 | 84             | 36 | 38 | 10 | 0.995 |
|              |      |                | Azoo  |        | 46          | 20 | 19 | 7  |                 |    |    |    |       |
|              |      |                | OAT   |        | 28          | 11 | 14 | 3  |                 |    |    |    |       |
| Weiner       | 2013 | 6              | Total | PB     | 271         | 129| 116| 26 | 301            | 153| 115| 33 | 0.112 |
|              |      |                | Azoo  |        | 98          | 49 | 41 | 8  |                 |    |    |    |       |
|              |      |                | oligo |       | 82          | 40 | 31 | 11 |                 |    |    |    |       |
| Naqvi        | 2014 | 5              | Total | NA     | 637         | 447| 154| 36 | 364            | 275| 79 | 10 | 0.145 |
|              |      |                | Azoo  |        | 49          | 34 | 11 | 4  |                 |    |    |    |       |
|              |      |                | OAT   |        | 65          | 39 | 24 | 2  |                 |    |    |    |       |
|              |      |                | oligo |       | 37          | 23 | 12 | 2  |                 |    |    |    |       |
| Mfady        | 2014 | 6              | Total | HB     | 150         | 67 | 63 | 20 | 150            | 74 | 67 | 9  | 0.221 |

(Continued)
examined by Z test and \( P \) (two-tailed) <0.05 was considered statistically significant. A chi-square test was used to examine the deviation from HWE for controls and \( P \) value<0.05 signified a departure from HWE. In this meta-analysis, heterogeneity between studies was assessed by the \( I^2 \) test and which was considered statistically significant with \( I^2 > 50\% \) [22]. A fixed (the Mantel-Haenszel method) [23] or random effects model (the DerSimonian and Laird method) [24] was used to calculate pooled effect estimates in the absence (\( I^2 / C^2 < 50\% \)) or presence (\( I^2 > 50\% \)) of heterogeneity, respectively. When heterogeneity between studies is absent, these two models provide similar results, if not, the random effects model is more appropriate. Subgroup analysis was performed by ethnicity, sperm concentration and the control sources. In addition, to assess the stability of results, sensitivity analysis was performed by omitting one study at a time and calculating the overall homogeneity and effect size. As publication bias was always concerned in meta-analysis, an evaluation of which was carried out with funnel plot and Egger’s test (\( P < 0.05 \) was significant publication bias) [25]. The statistical analysis was performed with STATA statistical software (Version 12.0; Stata Corporation, College Station, TX, USA).

**Results**

**Study characteristics**

Through a literature searching, initially a total of 46 potentially relevant publications were indentified. Out of these, eleven studies were eliminated because they did not investigate the association between the \( MTHFR \) 677C>T polymorphism and male infertility risk. After data extraction, we excluded 4 articles from the meta-analysis. One of them had no controls [26] while one of them had no cases [27], besides, the other two studies were excluded as one did not provide detailed information needed for OR calculation [28] while the last one did not directly research about the genotype and male infertility risk [29]. Hence, we obtained 31 relevant articles

| First author | Year | Quality scores | Group | Design | Cases | CC | CT | TT | Controls | CC | CT | TT | \( P \) HWE |
|--------------|------|----------------|-------|--------|-------|----|----|----|----------|----|----|----|-----------|
| Africans     |      |                |       |        |       |    |    |    |          |    |    |    |           |
| Eloualid     | 2012 | 5              | Total | NA     | 257   | 152 | 88 | 17 | 690      | 351 | 286 | 53 | 0.617     |
| Azoo         |      |                |       |        | 110   | 65  | 37 | 8  |          |     |     |    |           |
| oligo        |      |                |       |        | 147   | 87  | 51 | 9  |          |     |     |    |           |
| Not mentioned|      |                |       |        |       |    |    |    |          |    |    |    |           |
| Gava (37)    | 2011 | 7              | Total | PB     | 156   | 81  | 60 | 15 | 233      | 167 | 53  | 13 | 0.003     |
| Azoo         |      |                |       |        | 49    | 27  | 15 | 7  |          |     |     |    |           |
| oligo        |      |                |       |        | 107   | 54  | 45 | 8  |          |     |     |    |           |
| Gava (49)    | 2011 | 7              | Total | PB     | 133   | 66  | 51 | 16 | 173      | 136 | 27  | 10 | 0.000     |
| Azoo         |      |                |       |        | 55    | 35  | 14 | 6  |          |     |     |    |           |
| oligo        |      |                |       |        | 78    | 31  | 37 | 10 |          |     |     |    |           |
| Vani         | 2012 | 6              | Total | HB     | 206   | 158 | 42 | 6  | 230      | 188 | 42  | 0  | 0.128     |
| Camprubí     | 2013 | 6              | Total | PB     | 107   | 47  | 43 | 17 | 25       | 8   | 15  | 2  | 0.172     |

Azoo-azoospermia; OAT-oligoasthenoteratozoospermia; oligo-oligozoospermia

HB, hospital-based controls; PB, population-based controls

HWE, Hardy Weinberg Equilibrium

NA, not available

doi:10.1371/journal.pone.0121147.t001
| First author     | Year | Adequate definition of case | Representativeness of cases | Selection of control | Definition of control | Control for important factor or additional factor | Exposure assessment | Same method of ascertainment for cases and controls | Nonresponse rate | Total quality scores |
|------------------|------|-----------------------------|-----------------------------|----------------------|----------------------|-----------------------------------------------|---------------------|-----------------------------------------------|-----------------|---------------------|
| Ebisch           | 2003 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 7                   |
| Stuppia          | 2003 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 5                   |
| Park             | 2005 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 6                   |
| Singh            | 2005 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 6                   |
| Lee              | 2006 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 6                   |
| Paracchini       | 2006 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 6                   |
| A                | 2007 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 6                   |
| Sun              | 2007 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 6                   |
| Ravel            | 2009 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 6                   |
| Yang             | 2010 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 5                   |
| Gava (37)        | 2011 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 7                   |
| Gava (49)        | 2011 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 7                   |
| Safarinejad      | 2011 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 6                   |
| Murphy           | 2011 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 7                   |
| Qiu              | 2011 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 7                   |
| Gupta            | 2011 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 6                   |
| Eloualid         | 2012 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 5                   |
| Chellat          | 2012 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 5                   |
| Vani             | 2012 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 5                   |
| Liu              | 2012 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 6                   |
| Camprubí         | 2013 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 6                   |
| Pei              | 2013 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 7                   |
| Weiner           | 2013 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 6                   |
| Naqvi            | 2014 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 5                   |
| Mfady            | 2014 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 5                   |
| Li               | 2014 | ★                            | ★                           | ★                    | ★                    | ★                                             | ★                   |                                |                 | 6                   |

*aA study can be awarded a maximum of one star for each numbered item except for the item Control for important factor or additional factor.

bA maximum of two stars can be awarded for Control for important factor or additional factor.

doi:10.1371/journal.pone.0121147.t002
that examined the association between MTHFR 677C>T and male infertility risk. Among of them, five studies were excluded because of the poor quality, which was evaluated by Newcastle-Ottawa Scale [30–34]. Therefore, only 26 studies qualifying our strict selection criteria were involved in the meta-analysis [11, 16, 19, 35–57] (Fig. 1). We established a database of the extracted information from each eligible article (Table 1). The total data for this analysis included 5,575 cases and 5,447 controls for MTHFR 677C>T polymorphism. All the researches contained in this meta-analysis are case-control studies. Of the 26 studies included in the meta-analysis, there were 10 studies of Asians, 11 studies of Caucasians, 1 study of African and 4 studies did not mention. According to the control sources, general populations were used as controls in 12 studies whereas hospital patients were used in 8 studies and 6 studies did not mention. The genotype distributions among the controls of all studies followed HWE except for two studies [37, 49].

Overall analysis

MTHFR 677C>T polymorphism showed significant associations with male infertility risk in both fixed effects (CT+TT vs. CC: OR = 1.34, 95% CI: 1.23–1.46, \( P = 0.000, I^2 = 68.2\% \); TT vs. CT+CC: OR = 1.60, 95% CI: 1.41–1.81, \( P = 0.000, I^2 = 36.9\% \); TT vs. CC: OR = 1.76, 95% CI: 1.53–2.01, \( P = 0.000, I^2 = 54.0\% \); T vs. C: OR = 1.32, 95% CI: 1.24–1.41, \( P = 0.000, I^2 = 71.5\% \))
and random effects models (CT+TT vs. CC: OR = 1.39, 95% CI: 1.19–1.62, \( P = 0.000 \), \( I^2 = 68.2\% \), Fig. 2A; TT vs. CT+CC: OR = 1.58, 95% CI: 1.34–1.88, \( P = 0.000 \), \( I^2 = 36.9\% \); TT vs. CC: OR = 1.80, 95% CI: 1.44–2.24, \( P = 0.000 \), \( I^2 = 54.0\% \); T vs. C: OR = 1.36, 95% CI: 1.20–1.53, \( P = 0.000 \), \( I^2 = 71.5\% \)).

Ethnic origin

When stratified by ethnicity, the same associations between the MTHFR 677C>T polymorphism and male infertility were found in Asians (CT+TT vs. CC: fixed effects model, OR = 1.54, 95% CI: 1.35–1.76, \( P = 0.000 \), \( I^2 = 0\% \)) and Caucasians (CT+TT vs. CC: fixed effects model, OR = 1.19, 95% CI: 1.05–1.36, \( P = 0.007 \), \( I^2 = 48.1\% \), Fig. 2B).

Sperm concentration subgroup

Subgroup analyses were also performed for azoospermia (Azoo), oligoasthenoteratozoospermia (OAT) and oligozoospermia (oligo) groups (Fig. 2C). Studies without the genotype frequencies for these subgroups were excluded. Finally, the total number of cases and controls for Azoo, OAT and oligo were 1,412 and 3,532, 615 and 1,865, 1,006 and 2,490, respectively. The results showed enhanced risks of male infertility with the MTHFR 677C>T were acquired both in Azoo (CT+TT vs. CC: fixed effects model, OR = 1.36, 95% CI: 1.18–1.55, \( P = 0.000 \), \( I^2 = 49.1\% \)) and OAT (CT+TT vs. CC: fixed effects model, OR = 1.35, 95% CI: 1.11–1.64, \( P = 0.003 \).
$I^2 = 44.7\%$) subgroups. Whereas, no significant association was observed in oligo subgroup (CT+TT vs. CC: random effects model, OR = 1.34, 95% CI: 0.91–1.98, $P = 0.138$, $I^2 = 81.7\%$).

**Control sources**

When considered the sources of the control groups, five studies were excluded for unclear source of controls. Dramatically elevated risks were found both in population-based (CT+TT vs. CC: random effects model, OR = 1.66, 95% CI: 1.23–2.23, $P = 0.001$, $I^2 = 72.5\%$) and hospital-based controls (CT+TT vs. CC: fixed effects model, OR = 1.32, 95% CI: 1.15–1.52, $P = 0.000$, $I^2 = 0.0\%$) (Fig. 2D).

**Sensitivity analyses**

To assess the effect of individual study on all subjects and subgroups, the sensitivity analyses were performed by excluding one study each time. If the exclusion of any single study did not alter the significance of the final decision, it suggested that the outcomes were robust. However, our corresponding pooled ORs were not materially altered in all subjects and all subgroups. In addition, when excluding the two studies [37, 49] which did not follow HWE, the pooled ORs for all subjects still did not change remarkably (data not shown). Therefore, the results of sensitivity analyses confirmed the stability for our results in this meta-analysis.

**Publication bias**

For the diagnosis of publication bias, both Begg’s Funnel plot and Egger’s test were performed in this meta-analysis. The shape of the funnel plot was symmetrical and the $P$ value of Egger’s test also suggested that there was no evidence of publication bias ($P = 0.339$; Fig. 3).

![Fig 3. Funnel plot for publication bias of MTHFR 677C>T and infertility risk (CT+TT vs. CC) in overall populations. The horizontal line in the funnel plot indicates the random-effects summary estimate. Sloping lines indicate the expected 95% CI for a given SE.](https://doi.org/10.1371/journal.pone.0121147.g003)
**Discussion**

Male infertility is a heterogeneous disease, with various genetic and environmental factors contributing to the impairment of spermatogenesis. Spermatogenesis is a complex process that is governed by a tightly controlled series of gene expression events [58] and is associated with folate metabolism [59–61]. A possible candidate gene for genetic susceptibility to spermatogenic failure is MTHFR. MTHFR is an important regulatory enzyme in folate metabolism, DNA synthesis and remethylation reactions [62, 63]. It is suggested that the MTHFR might play an important role in spermatogenesis because of its higher activity in testes than in other major organs in the adult mouse [64]. In 2001, Bezold et al. [31] first reported the association between MTHFR 677C>T mutation and the male infertility risk. Subsequently, many epidemiological studies have been addressed to investigate the association between MTHFR 677C>T polymorphism and the risk of male infertility during the past decades, but the findings were inconsistent. Consequently, five meta-analyses [15–19] were conducted to examine the association between male infertility risk and the MTHFR 677C>T polymorphism, however, the results of them were controversial. In addition, the data of last meta-analysis [19] was updated two years ago (Apr. 2012). During these two years, many studies investigated the association between the MTHFR 677C>T polymorphism and male infertility risk were published. Therefore, in order to derive a powerful estimate of the male infertility risk associated with the MTHFR 677C>T polymorphism, we carried out the present meta-analysis.

The present meta-analysis, including 5,575 cases and 5,447 controls from 26 published studies, explored the association between the MTHFR 677C>T polymorphism and male infertility risk. The numbers of contained studies, the cases and controls in this meta-analysis are much more than the prior five meta-analyses. In addition, we did the quality assessment for the studies by Newcastle-Ottawa Scale while all the former meta-analyses did not do. Only the study had good quality of which score was more than 5 stars, then it could be included in the meta-analysis. Hence, our study made a more detailed and convincible evaluation than all the prior meta-analyses did. Overall, we find the MTHFR 677C>T variant genotype were significantly associated with male infertility risk based on both fixed effects and random effects models, and which was consistent with the results of Tüttelmann et al. [15], Gupta et al. [16] and Wu et al. [17], but inconsistent with the results of Wei et al. [18] and Weiner et al. [19]. All the characteristics and results of the present study compared with the former five meta-analyses were summarized in Table 3.

As heterogeneity for MTHFR 677C>T polymorphism among all the studies was significant, we conducted the subgroup analyses. When stratified by ethnicity, our results indicated that significant male infertility risks of people with MTHFR 677C>T polymorphism both in Asians and Caucasians. We are the first one that reported significant association between MTHFR 677C>T polymorphism and male infertility in Caucasians. Moreover, the heterogeneity was effectively decreased or removed after subgroup analyses by ethnicity. Therefore, as the differences of genetic backgrounds among different populations, one main reason for heterogeneity in this meta-analysis may be the ethnicity.

According to the sperm concentration, we found remarkable associations between Azoo or OAT subgroup and MTHFR 677C>T polymorphism in the present study. While, no strong association was observed in oligo subgroup. Compared with the former meta-analyses, we are the first one reported the MTHFR 677C>T polymorphism has a dramatically increased risk in male infertility susceptibility in OAT subgroup and the sensitivity analysis showed the stabilization of this result. However, as the extreme heterogeneity existed in oligo subgroup, the result of it may be less powerful and we should treat it carefully. Further studies based on oligo and MTHFR 677C>T are necessary to confirm the association.
Table 3. Characteristics and results of the present study compared with the former five meta-analyses.

| First author | Year of publication | Time of data updated | No. of studies | No. of cases | No. of controls | Quality assessment | Overall results | Asians | Caucasians | Azoo | OAT | Oligo | PB | HB |
|--------------|---------------------|----------------------|----------------|--------------|----------------|-------------------|----------------|--------|------------|------|-----|-------|----|----|
| Tüttelmann F | 2007                | NA                   | 8              | 1843         | 1791           | No                | +              | NA     | NA         | NA   | NA | NA     | NA | NA |
| Gupta N      | 2011                | Mar. 2011            | 14             | 3094         | 2877           | No                | +              | NA     | NA         | +    | −   | +      | NA | NA |
| Wu W         | 2012                | Sep. 2010            | 9              | 2275         | 1958           | No                | +              | +      | +          | −    | +  | −      | NA | NA |
| Wei B        | 2012                | Jan. 2011            | 11             | 2217         | 2312           | No                | −              | +      | −          | NA   | NA | NA     | NA | NA |
| Weiner AS    | 2014                | Apr. 2012            | 13             | 2972         | 3436           | No                | −              | NA     | NA         | −    | −  | −      | NA | NA |
| The present study | NA      | Jun. 2014            | 26             | 5575         | 5447           | Yes               | +              | +      | +          | +    | +  | +      | +  | +  |

Azoo-azoospermia; OAT-oligoasthenoteratozoospermia; Oligo-oligozoospermia
HB, hospital-based controls; PB, population-based controls
+, positive result
−, negative result
NA, not available

doi:10.1371/journal.pone.0121147.t003
In addition, when stratified by the sources of the control groups, our results showed that the \textit{MTHFR} 677C>T polymorphism were dramatic associated with male infertility risk both in population-based and hospital-based controls. At present, it is reported that the \textit{MTHFR} 677C>T polymorphism could influence the susceptibility to some common diseases, such as type 2 diabetes [65], coronary heart disease [66] and colorectal cancer, et al. [67]. Thus, the data on hospital-based controls may have a high risk of inducing unreliable result because it may not always represent the general population indeed, especially when the genotype under investigation are expected to affect other diseases. However, we obtained consistent results between population-based and hospital-based controls.

In recent years, the application of the genome-wide association study (GWAS) in many types of diseases has exploded. Until now, there are twelve GWASs about male infertility, and two of them [68, 69] mentioned the \textit{MTHFR} 677C>T polymorphism. However, concerned with the male infertility and \textit{MTHFR} 677C>T polymorphism, the results of these two GWASs were inconsistent with our meta-analysis. The reasons of this phenomenon are possibly as follows: (1) These two GWASs both conducted by Aston KI only included Caucasians of European decent, while we included men of other ethnicities. SNP frequencies vary widely between ethnic groups, for example, the frequency of the \textit{MTHFR} 677C>T allele varies significantly among populations, ranging from 30% to 40% in Europe and America to 5% to 10% in Africa and Sri Lanka [70, 71]; (2) The small sample size both of these two GWASs (Aston et al., 2009: 80 controls, 92 cases; Aston et al., 2010: 158 controls, 282 cases) limited the interpretation of the results. However, a large portion of the heritability of complex diseases has not been well explained by GWASs [72]. About missing heritability, one plausible explanation may be the loss of low-frequency variants, which are not well captured by current genotyping arrays [73]. Another explanation is that the common variants of small effect sizes, do not reach the thresholds of genome wide significance in GWASs [74].

Although several previous meta-analyses have researched the association between male infertility risk and \textit{MTHFR} 677C>T polymorphism, our study was more rigorous and comprehensive. First, more up-to-date studies (26 studies) that contained a substantial number of cases and controls were pooled from published studies, which dramatically increased the statistical power of the analysis. Second, the quality of studies included in this meta-analysis was satisfactory, as we first used the Newcastle-Ottawa Scale to assess the quality of the articles. When the study met our strict criteria, it could be enrolled in. Third, we are the first that showed the significantly increased risk between \textit{MTHFR} 677C>T polymorphism and male infertility in Caucasians and OAT subgroup. Also, for the first time we performed in detail to check into the association between male infertility risk and \textit{MTHFR} 677C>T polymorphism according to the control sources. Our results showed obvious higher male infertility risk in both population-based and hospital-based controls.

Nonetheless, there are some limitations should be addressed in this meta-analysis. First of all, the heterogeneities for \textit{MTHFR} 677C>T polymorphism among all the studies was dramatic. Even though we conducted subgroup analyses and the heterogeneity was efficiently decreased or removed in some subgroups, it still existed in the population-based controls and oligo subgroup. Several reasons could explain the significant heterogeneity. First, lifestyle factors especially intake of folate and B-vitamins may play a role. For instance, deficient intake of folate and vitamin B12 is prevalent in India [75–77]; in contrast, sufficient intake of folate is prevalent in France [78], Korea [11] and Italy [79]. Second, due to the various genotyping methods used in the studies, the genotyping error is hard to avoid. However, when we eliminated the studies which did not follow the HWE, our results did not change statistically. Third, it has been shown that there were other underlying confounding factors in the included studies, such as gene-environment interaction, or selection bias, or chance [80, 81]. Although evidence
of heterogeneity exists, our overall results used by fixed effects models were consistent with which used by random effects models. Moreover, the sensitivity analyses results showed that all of our results were stable and certain. Secondly, as the use of unadjusted data, some potential confounding factors, such as age, sex and residence might slightly modify the effective estimates. Hence, a more precise evaluation according to the adjusted data is needed. Finally, as only published studies were retrieved in the present meta-analysis, the selection bias may have occurred, even though the funnel plot and Egger’s test have not shown publication bias. Also, the number of published studies was not large enough for a comprehensive analysis, particularly for some subgroups.

Conclusions
In summary, our meta-analysis suggested that the MTHFR 677C>T polymorphism is associated with an enhanced risk of male infertility, supporting the hypothesis that the MTHFR 677C>T may be a potential cause of male infertility. Specifically, increased infertility risks with MTHFR 677C>T were observed in overall analysis and most subgroups, except oligo subgroup. However, large scale, well-designed and high quality epidemiological studies will be required to confirm our findings in the future.

Supporting Information
S1 Checklist. (DOC)
(DOCX)

Author Contributions
Conceived and designed the experiments: RQY SPQ. Performed the experiments: MCG WJD. Analyzed the data: GYH RR SCH. Contributed reagents/materials/analysis tools: TYH ZRS. Wrote the paper: MCG WJD RQY SPQ.

References
1. O’Flynn O’Brien KL, Varghese AC, Agarwal A. The genetic causes of male factor infertility: a review. Fertil Steril. 2010; 93: 1–12. doi: 10.1016/j.fertnstert.2009.10.045 PMID: 20103481
2. Dowsing AT, Yong EL, Clark M, McLachlan RI, de Kretser DM, Trounson AO. Linkage between male infertility and trinucleotide repeat expansion in the androgen-receptor gene. Lancet. 1999; 354: 640–643. PMID: 10466666
3. Ferlin A, Raicu F, Gatta V, Zuccarello D, Paika G, Foresta C. Male infertility: role of genetic background. Reprod Biomed Online. 2007; 14: 734–745. PMID: 17579990
4. Foresta C, Moro E, Ferlin A. Y chromosome microdeletions and alterations of spermatogenesis. Endocr Rev. 2001; 22: 226–239. PMID: 11294825
5. Dohle GR, Halley DJ, Van Hemel JO, van den Ouwel AM, Pieters MH, Weber RF, et al. Genetic risk factors in infertile men with severe oligozoospermia and azoospermia. Hum Reprod. 2002; 17: 13–16. PMID: 11756355
6. Bashamboo A, Ferraz-de-Souza B, Lourenço D, Lin L, Sebire NJ, Montjéan D, et al. Human male infertility associated with mutations in NR5A1 encoding steriodogenic factor 1. Am J Hum Genet. 2010; 87: 505–512. doi: 10.1016/j.ajhg.2010.09.009 PMID: 20887963
7. Donnelly JG. Folic acid. Crit Rev Clin Lab Sci. 2001; 38: 183–223. PMID: 11451208
8. Fowler B. Homocysteine: overview of biochemistry, molecular biology, and role in disease processes. Semin Vasc Med. 2005. 5: 77–86. PMID: 16047261
9. Li E. Chromatin modification and epigenetic reprogramming in mammalian development. Nat Rev Genet. 2002; 3: 662–673. PMID: 12209141
10. Kobayashi H, Sato A, Otsu E, Hiura H, Tomatsu C, Utsunomiya T, et al. Aberrant DNA methylation of imprinted loci in sperm from oligospermic patients. Hum Mol Genet. 2007; 16: 2542–2551. PMID: 17636251
11. Lee HC, Jeong YM, Lee SH, Cha KY, Song SH, Kim NK, et al. Association study of four polymorphisms in three folate-related enzyme genes with non-obstructive male infertility. Hum Reprod. 2006; 21: 3162–3170. PMID: 16861746

12. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet. 1995; 10: 111–113. PMID: 7647779

13. Goyette P, Pai A, Milos R, Frosst P, Tran P, Chen Z, et al. Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR). Mamm Genome. 1998; 9: 652–656. PMID: 9680386

14. Austin RC, Lentz SR, Werstuck GH. Role of hyperhomocysteinemia in endothelial dysfunction and atherothrombotic disease. Cell Death Differ. 2004; 11: S56–S64. PMID: 15243582

15. Tüttelmann F, Raipert-De Meyts E, Nieschlag E, Simoni M. Gene polymorphisms and male infertility— a meta-analysis and literature review. Reprod Biomed Online. 2007; 15: 643–658. PMID: 18062861

16. Gupta N, Gupta S, Dama M, David A, Khanna G, Khanna A, et al. Strong association of 677C>T substitution in the MTHFR gene with male infertility—a study on an indian population and a meta-analysis. PLoS One. 2011; 6: e22277. doi: 10.1371/journal.pone.0022277 PMID: 21799811

17. Wu W, Shen O, Qin Y, Lu J, Niu X, Zhou Z, et al. Methylenetetrahydrofolate reductase C677T polymorphism and the risk of male infertility: a meta-analysis. Int J Androl. 2012; 35: 18–24. doi: 10.1111/j.1365-2605.2011.01147.x PMID: 21535009

18. Wei B, Xu Z, Ruan J, Zhu M, Jin K, Zhou D, et al. MTHFR 677C>T and 1298A>C polymorphisms and male infertility risk: a meta-analysis. Mol Biol Rep. 2012; 39: 1997–2002. doi: 10.1007/s11033-011-0946-4 PMID: 21643754

19. Weiner AS, Boyarskikh UA, Voronina EN, Tupikin AE, Korolkova OV, Morozov IV, et al. Polymorphisms in folate-metabolizing genes and risk of idiopathic male infertility: a study on a russian population and a meta-analysis. Fertil Steril. 2014; 101: 87–94.e3. doi: 10.1016/j.fertnstert.2013.09.014 PMID: 24268703

20. GA Wells, B Shea, D O’Connell, J Peterson, V Welch, M Losos, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in the meta-analysis. Available: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp Accessed November 6, 2013.

21. Owmby RL, Crocco E, Acevedo A, John V, Loewenstein D. Depression and risk for Alzheimer disease: systematic review, meta-analysis. Arch Gen Psychiatry. 2006; 63: 530–538. PMID: 16651510

22. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003; 327: 557–560. PMID: 12958120

23. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst. 1959; 22: 719–748. PMID: 13655060

24. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986; 7: 177–188. PMID: 3802833

25. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. Br Med J. 1997; 315: 629–634. PMID: 9310563

26. Suryandari DA, Wiweko B, Yunaini L. Genotype distribution of methylenetetrahydrofolate reductase A1298C and C677T gene in Indonesian infertile men. Med J Indones. 2012; 21: 23.

27. A ZC, Zhang W. Effect of Polymorphism of 677C/T in MTHFR Gene on Sperm Count. Reproduction & Contraception. 2010; 3: 183–185.

28. Lee S, Jeong YM, Lee SK, Cha KY, Chung TG, Yoon TK, et al. The 677 C>T polymorphism in methylenetetrahydrofolate reductase (MTHFR) gene associates with unexplained male infertility with severe OAT. Fertil Steril. 2003; 80: S229.

29. Montjean D, Benkhalfa M, Dessolle L, Cohen-Bacrie P, Belloc S, Siffroi JP, et al. Polymorphisms in MTHFR and MTRR genes associated with blood plasma homocysteine concentration and sperm counts. Fertil Steril. 2011; 95: 635–640. doi: 10.1016/j.fertnstert.2010.08.054 PMID: 20885556

30. Dhillon VS, Shahid M, Husain SA. Associations of MTHFR DNMT3b 4977bp deletion in mtDNA and GSTM1 deletion, and aberrant CpG island hypermethylation of GSTM1 in non-obstructive Infertility in Indian men. Mol Hum Reprod. 2007; 13: 213–222. PMID: 17277043

31. Bezold G, Lange M, Peter RU. Homozygous methylenetetrahydrofolate reductase C677T mutation and male infertility. N Engl J Med. 2001; 344: 1172–1173. PMID: 11302150

32. Tetik A, Aliyeva U, Cetintas VB, Semerci B, Topcuoglu N, Erdogan Z. Influence of methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C gene polymorphisms on male infertility in turkish infertile men with azoospermia and oligozoospermia. Eur Urol. 2008; Suppl 7 : 92.
33. Dordević Valentina, Nikolić A, Ljubić M, Nestorović A, Ristanović M, Tulic C, et al. Combined effect of GSTM1 gene deletion, GSTT1 gene deletion and MTHFR C677T mutation in male infertility. Archives of Biological Sciences. 2010; 62: 525–530.

34. Kumar K, Venkatesh S, Sharma PR, Tiwari PK, Dada R. DAZL 260A>C and MTHFR 677C>T variants in sperm DNA of infertile Indian men. Indian J Biochem Biophys. 2011; 48: 422–426. PMID: 22329245

35. Ebisch IMW, van Heerde WL, Thomas CM, van der Put N, Wong WY, Steegers-Teunissen RP. C677T methylenetetrahydrofolate reductase polymorphism interferes with the effects of folic acid and zinc sulfate on sperm concentration. Fertil Steril. 2003; 80: 1190–1194. PMID: 14607573

36. Ravel C, Chantot-Bastaraud S, Chalmy C, Barreiro L, Aknin-Seifer I, Pfeffer J, et al. Lack of association between genetic polymorphisms in enzymes associated with folate metabolism and unexplained reduced sperm counts. Plos One. 2009; 4: e6540. doi: 10.1371/journal.pone.0006540 PMID: 19657388

37. Gava MM, Chagas Ede O, Bianco B, Christofolini DM, Pompeo AC, Giлина S, et al. Methylenetetrahydrofolate Reductase Polymorphisms Are Related to Male Infertility in Brazilian Men. Genet Test Mol Biomarkers. 2011; 15: 153–157. doi: 10.1089/gtmb.2010.0128 PMID: 21138341

38. Park JH, Lee HC, Jeong YM, Chung TG, Kim HJ, Kim NK, et al. MTHFR C677T polymorphism associates with unexplained infertile male factors. J Assist Reprod Genet. 2005; 22: 361–368. PMID: 16247718

39. Paracchini V, Garte S, Tacioli E. MTHFR C677T polymorphism, GSTM1 deletion and male infertility: a possible suggestion of a gene-gene interaction? Biomarkers. 2006; 11: 53–60. PMID: 16484136

40. Singh K, Singh SK, Sah R, Singh I, Raman R. Mutation C677T in the methylenetetrahydrofolate reductase gene is associated with male infertility in Indian population. Int J Androl. 2005; 28: 115–119. PMID: 15811073

41. Safarinejad MR, Shafiei N, Safarinejad S. Relationship Between Genetic Polymorphisms of Methylenetetrahydrofolate Reductase (C677T, A1298C, and G1793A) as Risk Factors for Idiopathic Male Infertility. Reprod Sci. 2011; 18: 304–315. doi: 10.1177/1933719110385135 PMID: 20978181

42. A ZC, Yang Y, Zhang SZ, Li N, Zhang W. Single nucleotide polymorphism C677T in the methylenetetrahydrofolate reductase gene might be a genetic risk factor for infertility for Chinese men with azoosperma or severe oligozoosperma. Asian J Androl. 2007; 9: 57–62. PMID: 16886882

43. Stuppa L, Gatta V, Scarciolla O, Colosimo A, Guanciali-Franchi P, Calabrese G, et al. The methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and male infertility in Italy. J Endocrinol Invest. 2003; 26: 620–622. PMID: 14594111

44. Murphy LE, Mills JL, Molloy AM, Qian C, Carter TC, Strevens H, et al. Folate and vitamin B12 in idiopathic male infertility. Asian journal of andrology. 2011; 13: 856–861. doi: 10.1038/aja.2011.96 PMID: 21857689

45. Eloualid A, Abidi O, El Houate B, Benrahma H, Louanjli N, et al. Association of the MTHFR A1298C variant with unexplained severe male infertility. PloS one. 2012; 7: e34111. doi: 10.1371/journal.pone.0034111 PMID: 22457816

46. Chellat D, Rezgoune ML, Hamane D, Semmame O, Benlatrò Đ. Lack of association of Vani GT, Mukesh N, Rama Devi P, Usha Rani P, Reddy PP. Methylenetetrahydro folate reductase C677T polymorphism is not associated with male infertility in a South Indian population. Andrologia. 2012; 44: 252–259. doi: 10.1111/j.1439-0272.2011.01172.x PMID: 21729137

47. Gava MM, Kayaki EA, Blanco B, Teles JS, Christofolini DM, Pompeo AC, et al. Polymorphisms in Folate-Related Enzyme Genes in Idiopathic Infertile Brazilian Men. Reproductive Sciences. 2011; 18: 1267–1272. doi: 10.1177/19337191114111729 PMID: 21775772

48. Liu L, Cai Z, Leng HM, Qian WP. Association of MTHFR C677T and MS A2756G polymorphism with semen quality. J Cent South Univ (Med Sci). 2011; 37: 1054–1059.

49. Pei J. Association between MTHFR C677T Polymorphism and Male Infertility in Han Population of He Nan China. China Hwalth Care & Nutrition. 2013; 7: 629–630.

50. Qiu XF, Hu XP, Li YJ, Dang J, Peng L, Xu X, et al. Association between Methylenetetrahydrofolate Reductase Gene C677 T Polymorphism and Male Infertility with Azoosperma or Severe oligozoosperma
and Asthenospermia in Ningxia Han Population. Journal of Ningxia Medical University. 2011; 33: 625–628.

53. Yang BH, Peng GF, Pi JP. Association of methylenetetrahydrofolate reductase gene C677T polymorphism with asthenospermia in Han population of South Anhui. J of Wannan Medical University. 2010; 29: 5–7.

54. Sun HT, Zhang JY, Lu YJ. Associated of the Methylenetetrahydrofolate Reductase Gene C677T Polymorphism with Male Infertility. Reproduction & Contraception. 2007; 7: 434–446.

55. Naqvi H, Hussain SR, Ahmad MK, Mahdi F, Jaiswar SP, Shankwarf SN, et al. Role of 677C>T polymorphism a single substitution in methylenetetrahydrofolate reductase (MTHFR) gene in North Indian infertile men. Mol Biol Rep. 2014; 41:573–579. doi: 10.1007/s11033-013-2894-7 PMID: 24366618

56. Mfady DS, Sadiq MF, Khabour OF, Fararjeh AS, Abu-Awad A, Khader Y. Associations of variants in MTHFR and MTRR genes with male infertility in the Jordanian population. Gene. 2014; 536:443–446. doi:10.1016/j.gene.2013.12.001 PMID: 24334125

57. Li SS, Li J, Xiao Z, Ren AG, Jin L. Prospective study of MTHFR genetic polymorphisms as a possible etiology of male infertility. Genet Mol Res. 2014; 13(3):6367–6374. doi:10.4238/2014.March.24.26 PMID: 24737513

58. Maclean JA, Wilkinson MF. Gene regulation in spermatogenesis. Curr Top Dev Biol. 2005; 71: 131–197. PMID: 16344105

59. Singh K, Jaiswal D. One-carbon metabolism, spermatogenesis, and male infertility. Reprod Sci. 2013; 20: 622–630. doi: 10.1177/1933719112459232 PMID: 23138010

60. Bentivoglio G, Melica F, Cristoforoni P. Folinic acid in the treatment of human male infertility. Fertil Steril. 1993; 60: 698–701. PMID: 8405528

61. Wong WY, Merkus HMWM, Thomas CMG, Menkveld R, Zielhuis GA, Steegers-Theunissen RP. Effects of folic acid and zinc sulfate on male factor subfertility: a double-blind, randomized, placebo-controlled trial. Fertil Steril. 2002; 77: 491–498. PMID: 11872201

62. Stevenson RE, Schwartz CE, Du YZ, Adams MJ Jr. Differences in methylenetetrahydrofolate reductase genotype frequencies, between Whites and Blacks. Am J Hum Genet. 1997; 60: 229–230. PMID: 8981967

63. Schneider JA, Rees DC, Liu YT, Clegg JB. Worldwide distribution of a common methylenetetrahydrofolate reductase mutation. Am J Hum Genet. 1998; 62: 1258–1260. PMID: 9545406

64. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. Nature. 2009; 461:747–753. doi: 10.1038/nature08494 PMID: 19812666

65. Marth GT, Yu F, Indap AR, Garimella K, Gravel S, Leong WF, et al. The functional spectrum of low-frequency coding variation. Genome Biol. 2011; 12:R84. doi: 10.1186/gb-2011-12-9-r84 PMID: 21917140
74. Yang J, Benyamin B, McEvoy BP, Gordon S, Henders AK, Nyholt DR, et al. Common SNPs explain a large proportion of the heritability for human height. Nat Genet. 2010; 42:565–569. doi:10.1038/ng.608 PMID: 20562875

75. Pathak P, Saxena R, Kapoor SK, Dwivedi SN, Singh R, Kapil U. Status of serum ferritin and folate levels amongst young women in a rural community of Haryana, India. Nepal Med Coll J. 2004; 6: 13–16. PMID: 15449646

76. Antony AC. Prevalence of cobalamin (vitamin B-12) and folate deficiency in India—audi alteram partem. Am J Clin Nutr. 2001; 74: 157–159. PMID: 11470714

77. Lakshmi AV, Maniprabha C, Krishna TP. Plasma homocysteine level in relation to folate and vitamin B6 status in apparently normal men. Asia Pac J Clin Nutr. 2001; 10: 194–196. PMID: 11708307

78. Parodi PW. The French paradox unmasked: the role of folate. Med Hypotheses. 1997; 49: 313–318. PMID: 9352501

79. Pelucchi C, Meregalli M, Talamini R, Negri E, Montella M, Ramazzotti V, et al. Dietary folate, alcohol consumption, and risk of ovarian cancer in an Italian case-control study. Cancer Epidemiol Biomarkers Prev. 2005; 14: 2056–2058. PMID: 16103462

80. Zintzaras E, Ioannidis JP. Heterogeneity testing in meta-analysis of genome searches. Genet Epidemiol. 2005; 28: 123–137. PMID: 15593093

81. Lin PI, Vance JM, Pericak-Vance MA, Martin ER. No gene is an island: the flip-flop phenomenon. Am J Hum Genet. 2007; 80: 531–538. PMID: 17273975