Methylenetetrahydrofolate Reductase C677T Polymorphism and Risk for Male Infertility in Asian Population

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Received: 23 November 2016 / Accepted: 24 January 2017 / Published online: 8 February 2017
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Abstract Methylenetetrahydrofolate reductase (MTHFR) is a critical enzyme of folate pathway and required for DNA synthesis and methylation. MTHFE C677T polymorphisms is reported as risk factors for various diseases and disorders like birth defects, metabolic, neurological, psychiatric disorders, and cancers. Several studies have investigated association between the MTHFR C677T polymorphism and male infertility. To assess the risk associated with MTHFR C677T polymorphism in Asian population, a meta-analysis was performed. Included articles were collected from the following electronic databases: PubMed, Google Scholar, and Science direct up to March 2015. Risk was estimated as pooled odds ratios (ORs) with confidence intervals (CIs) for assessment. Seventeen case-control studies involving 4392 breast infertile males and 3667 fertile males were found suitable for the inclusion in the present meta-analysis. Results showed that the C677T polymorphism was significantly associated with male infertility in Asian population using all the five genetic models ($OR_{T \text{ vs. } C}$ (allele contrast model) = 1.86, 95% CI 1.7–2.0; $OR_{TT \text{ vs. } CC}$ (homozygote model) = 1.96, 95% CI 1.67–2.30; $OR_{CT \text{ vs. } CC}$ (co-dominant model) = 1.40, 95% CI 1.18–1.62; $OR_{TT+CT \text{ vs. } CC}$ (dominant model) = 1.53, 95% CI 1.30–1.77; $OR_{TT \text{ vs. } CT+CC}$ (recessive model) = 1.67, 95% CI 1.44–1.92). In conclusion, results of present meta-analysis strongly supported an association between C677T polymorphism and male infertility in Asians.

Keywords Male infertility · Polymorphism · MTHFR · C677T · Meta-analysis

Introduction

Infertility is very common reproductive problem, which affects approximately 15–20% of couples worldwide. Failure in conceiving a child after 1 year of regular unprotected intercourse is known as infertility. About 50% cases of infertility are due to male factors, especially defect in sperms or semen quality i.e. low sperm count [1–3]. Male infertility is a multifactorial clinical disorder with genetic as well as environmental causes. Several genetic risk factors for male infertility have been reported like aneuploidies, chromosomal translocation, deletions especially Y chromosome microdeletions and gene mutations [4]. About 50% cases of male infertility are idiopathic [3, 5].

Folates are a group of inter-convertible coenzymes, involved in DNA synthesis, methylation and amino acid metabolism. Folate deficiency and related hyperhomocysteinemia is reported as a risk factor for several diseases and disorders including male infertility. Spermatogenesis is a very complex event which is controlled by several genes present on autosomes and Y chromosome [6]. Folate deficiency and polymorphisms of folate pathway genes can influence DNA methylation and gene expression and may contribute to infertility [7].

Methylenetetrahydrofolate reductase (MTHFR) is a crucial enzyme of folate/homocysteine metabolic pathway. MTHFR reduces the 5–10-methylenetetrahydrofolate to its biologically active form 5-methyltetrahydrofolate, which donates its methyl group for the conversion of homocysteine into methionine [8]. Several polymorphisms in

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MTHFR gene have been reported [9], out of which only two C677T and A1298C polymorphisms are clinically important and most studied [10]. Frequency of C677T polymorphism varies greatly worldwide [11, 12]. C677T polymorphism is associated with DNA hypomethylation [9] and hyperhomocysteinemia [13]. The MTHFR gene variant is reported to be associated with the risk of certain human diseases, including Down syndrome, congenital heart defects, and pregnancy complications, such as pre-eclampsia, abruptio placentae, intrauterine growth retardation, preterm birth and intrauterine fetal death etc. [14–17].

Several case control association studies have reported that MTHFR gene variants is associated with reduced sperm counts in the human leading to male infertility in some populations [18–23]. During past two decade several case control studies regarding assessment of MTHFR polymorphism as risk factor for male infertility were published and reported controversial results. The aim of the present meta-analysis was to review results of these studies and find out the estimate of association between MTHFR C677T polymorphism and male infertility.

Methods

Meta-analysis was carried out according to MOOSE guidelines [24].

Search Strategy and Identification of Studies

Eligible studies were identified by searching PubMed, Google Scholar, Elsevier and Springer link databases. Following search terms were used: “MTHFR”, “methylenetetrahydrofolate reductase”, “C677T”, and “polymorphism” in combination with “male infertility” up to March 2015.

Inclusion and Exclusion Criteria

The inclusion criteria were as follows: studies should: 1) be original and published, 2) used case control approach and evaluated association between MTHFR C677T polymorphism and male infertility and 3) be reported genotype number/frequency of cases and controls, and if not, the text provided data enabling such calculations.

Studies were excluded if: (1) their sample was not independent from that investigated in another study, (2) incomplete raw data/information and not providing complete information for number of genotype and/or allele number calculation, (3) studies based on pedigree and (4) review, letter to editors and book chapters. If more than one study was published using the same dataset, the most recent

study, or the study with the larger sample size, was selected.

Data Extraction

The following information was extracted from the each identified studies: the first author family name, year of publication, sample size, country name, ethnicity, genotyping method, the numbers of patients and controls, and MTHFR C677T genotypes information and frequencies of alleles in all study. If important information was not given in the article, the relevant information was obtained by contacting authors.

Statistical Analysis

The strength of association between the C677T polymorphism of MTHFR and autism risk was estimated by the odds ratio (OR) along with its 95% confidence interval (CI). Heterogeneity among studies was examined with the χ^2 test-based Q statistics [25] and $p < 0.05$ was considered significant. To quantify heterogeneity, ‘ I^2 ’ index was used, which calculated as the percentage of the total variability in a set of effect sizes due to true heterogeneity [26] and ‘ I^2 ’ $> 50\%$ is considered high heterogeneity [27]. Fixed and random-effects summary ORs were calculated using the Mantel–Haenszel and DerSimonian and Laird methods [28, 29], random-effects summary ORs was used, when there is higher heterogeneity. The pooled ORs were performed for the allele contrasts/additive model (T vs. C), homozygote model (TT vs. CC), recessive model (TT vs. CT + CC), dominant model (TT + CT vs. CC), and co-dominant/heterozygote model (CT vs. CC). Control population of each study was tested for Hardy–Weinberg Equilibrium (HWE) using the χ^2 test.

Sensitivity analysis was performed to evaluate the stability of the results by removing the studies not in Hardy–Weinberg equilibrium (HWE), and studies with small sample size. Cumulative meta-analysis was performed to see the effect of subsequent addition of each study. The publication bias was evaluated by a Begg’s test and Egger’s linear regression test [30, 31]. All statistical analysis was undertaken using the program MIX version 1.7 [32]. P values were two-tailed with a significance level of 0.05.

Results

Characteristics of Included Studies

Seventeen studies were found suitable for the inclusion in the present meta-analysis [3, 7, 20, 22, 33–45]. The studies

were published between 2005 and 2015. The number of cases varied from 75 [41] to 637 [3], and the number of controls varied from 52 [35] to 396 [22]. The studies were carried out in China [34–36, 38, 41, 42, 44, 45], India [3, 7, 33, 37, 40], Iran [39, 43] and Korea [20, 22].

In all seventeen studies, total number of infertile cases were 4392 with CC (1,961), CT (1,714) and TT (717), and number of fertile controls were 3667 with CC (1,919), CT (1,362), and TT (386; Table 1). In case genotypes percentage of CC, CT and TT were 44.65, 39.0 and 16.32% respectively. In controls genotype percentages of CC, CT and TT were 52.33, 37.14 and 10.53% respectively. Frequencies of CC genotype and C allele were highest in both cases and controls.

Meta-Analysis

Table 2 summarizes the ORs with corresponding 95% CIs for association between MTHFR C677T polymorphism and risk of male infertility in allele contrast, dominant, recessive, homozygote and co-dominant models. Meta-analysis with allele contrast showed significant association between 677T allele and male infertility with both fixed effect ($OR_{T \text{ vs. } C} = 1.86$; 95% CI 1.7–2.0; $p < 0.0001$) and random effect model ($OR_{T \text{ vs. } C} = 1.99$; 95% CI 1.58–2.51; $p < 0.0001$; Fig. 1).

An increased significant association was found between male infertility and mutant genotype (TT vs. CC; homozygote model) with both fixed ($OR_{TT \text{ vs. } CC} = 1.96$; 95% CI 1.67–2.30, $p < 0.0001$) and random ($OR_{TT \text{ vs. } CC} = 2.1$;

95% CI 1.61–2.61, $p < 0.0001$) effect models (Table 2; Fig. 2). Association of mutant heterozygous genotype (CT vs. CC; co-dominant model) was observed significant with male infertility using fixed ($OR_{CT \text{ vs. } CC} = 1.40$; 95% CI 1.24–1.52; $p < 0.0001$) and random ($OR_{CT \text{ vs. } CC} = 1.40$; 95% CI 1.18–1.62; $p < 0.0001$) effect models (Table 2). Combined mutant genotypes (TT + CT vs. CC; dominant model) showed positive association with male infertility using both fixed ($OR_{TT+CT \text{ vs. } CC} = 1.50$; 95% CI 1.35–1.65; $p < 0.0001$) and random ($OR_{TT+CT \text{ vs. } CC} = 1.53$; 95% CI 1.30–1.77; $p < 0.0001$) effect models. Similarly the recessive genotypes model (TT vs. CT + CC) also showed positive association fixed ($OR_{TT \text{ vs. } CT+CC} = 1.67$; 95% CI 1.44–1.92; $p < 0.0001$) and random ($OR_{TT \text{ vs. } CT+CC} = 1.70$; 95% CI 1.38–2.1; $p < 0.0001$) effect models (Table 2).

A true heterogeneity existed between studies for allele contrast ($P_{\text{heterogeneity}} < 0.0001$, $Q = 151.38$, $I^2 = 89.43\%$, $t^2 = 0.2098$, $z = 5.80$), co-dominant ($P_{\text{heterogeneity}} = 0.005$, $Q = 33.80$, $I^2 = 52.67\%$, $t^2 = 0.055$, $z = 4.08$), and dominant ($P_{\text{heterogeneity}} = 0.005$, $Q = 34.54$, $I^2 = 53.68\%$, $t^2 = 0.05$, $z = 5.50$) comparisons.

Publication Bias

Except homozygote (Egger's $p = 0.02$) and recessive models, publication bias was not observed in three genetic models (Egger's $p = 0.21$ for T vs. C; Egger's $p = 0.59$ for CT vs. CC; Egger's $p = 0.28$ for TT + CT vs. CC; Table 2; Fig. 3).

Table 1 Characteristics of seventeen studies included in the present meta-analysis

References	Country	Case/control	Case genotypes CC/CT/TT	Control genotypes CC/CT/TT	<i>p</i> value (HWE)	Quality score
Singh et al. [33]	India	151/200	105/40/6	163/37/0	0.15	6
Park et al. [22]	Korea	373/396	105/205/63	145/200/51	0.16	6
Lee et al. [20]	Korea	360/225	115/181/64	118/66/41	0.00	6
Yang et al. [34]	China	355/252	130/160/65	128/95/29	0.08	6
Dhillon et al. [7]	India	179/200	81/77/21	70/100/30	0.55	5
Sun et al. [35]	China	182/53	27/86/69	15/28/10	0.63	5
Yang et al. [36]	China	131/293	34/55/42	98/142/53	0.90	5
Gupta et al. [37]	India	522/315	378/116/28	251/58/6	0.22	5
Qiu et al. [38]	China	271/180	75/112/84	63/85/32	0.72	6
Safarinejad et al. [39]	Iran	164/328	58/80/26	144/148/36	0.82	6
Vani et al. [40]	India	206/230	158/42/6	188/42/0	0.13	6
Liu et al. [41]	China	75/72	27/38/10	40/28/4	0.75	7
Pei [42]	China	290/90	39/138/113	24/47/19	0.65	7
Karimian and Colagar [43]	Iran	118/132	51/59/8	77/52/3	0.08	6
Li et al. [44]	China	82/133	14/36/32	36/61/36	0.34	6
Naqvi et al. [3]	India	637/364	447/154/36	275/79/10	0.144	5
Ni et al. [45]	China	296/204	117/135/44	84/94/26	0.97	5

Table 2 Summary estimates for the odds ratio (OR) of MTHFR C677T in various allele/genotype contrasts, the significance level (p value) of heterogeneity test (Q test), and the I^2 metric and publication bias p value (Egger test)

Genetic models	Fixed effect OR (95% CI), p	Random effect OR (95% CI), p	Heterogeneity p value (Q test)	I^2 (%)	Publication bias (p value of Egger's test)
Allele Contrast (T vs. C)	1.86 (1.7–2.00), <0.0001	1.99 (1.58–2.51), <0.0001	<0.0001	89.43	0.21
Co-dominant (CT vs. CC)	1.4 (1.24–1.52), <0.0001	1.40 (1.18–1.62), <0.0001	0.005	52.67	0.59
Homozygote (TT vs. CC)	1.96 (1.67–2.30), <0.0001	2.1 (1.61–2.61), <0.0001	0.02	47.35	0.02
Dominant (TT + CT vs. CC)	1.5 (1.35–1.65), <0.0001	1.53 (1.3–1.77), <0.0001	0.005	53.68	0.28
Recessive (TT vs. CT + CC)	1.67 (1.44–1.92), <0.0001	1.70 (1.38–2.1), <0.0001	0.03	43.73	0.014

Fig. 1 Random effect forest plot of allele contrast model (T vs. C) of *MTHFR* C677T polymorphism

Discussion

MTHFR plays an important role in folate and homocysteine metabolism, and tHcy levels could affect DNA synthesis and methylation. DNA methylation and DNA synthesis have important effects on spermatogenesis [46–48]. There are several experimental evidences that key enzymes of folate pathway are essential for spermatogenesis and gene polymorphism of these enzymes especially MTHFR coupled with folate deficiency can alter nucleotide synthesis and may cause infertility.

The role of MTHFR C677T polymorphism in male infertility is not clearly understood but it may be explained

by homocysteine hypothesis. Variant MTHFR enzyme less efficiently convert homocysteine into methionine and increase concentration of homocysteine [13]. Methionine is essential for the synthesis of S-adenosylmethionine (SAM), which is the main methyl donor for DNA methylation. Adequate SAM is required for cellular methylation reactions like DNA, RNA, protein and lipid methylation. C677T polymorphism causes global DNA hypomethylation. DNA hypomethylation affected expression of genes involved in spermatogenesis [49]. Further higher concentration of homocysteine increased oxidative stress and causes DNA damage [50] and mis-incorporation of uracil in DNA repair during spermatogenesis. Sperm chromatin

Fig. 2 Random effect forest plot of homozygote model (TT vs. CC) of *MTHFR* C677T polymorphism

Fig. 3 Funnel plots of precision by log odds ratio for allele contrast model

structure and DNA integrity have a critical role in the fertilization process [7]. In addition, hyperhomocysteinemia also reduced testicular blood flow due to vascular disease [10, 13, 51, 52].

Meta-analysis is the statistical analysis of a large collection of analysis results for the purpose of integrating the findings and it is a powerful tool for systematic review of a focused topic in the literature that provides a quantitative

estimate for the effect of a gene [53]. Several meta-analyses illustrate the utility of meta-analytic techniques in identification of risk association of folate pathway gene polymorphism with disease/disorders like- Down syndrome [54, 55], neural tube defects [56], orofacial clefts [57], recurrent pregnancy loss [58], psychiatric diseases [59–62], Alzheimer's disease [63], breast cancer [64–66], lung cancer [67] and prostate cancer [68] etc.

Finally, despite the clear strengths of present meta-analysis, including relatively large sample sizes and lack of publication bias, the interpretation should be done in light of few limitations like- (1) used crude ORs in the pooled analysis without adjustment, (2) some studies with small sample sizes were also included, (3) presence of significant heterogeneity in overall meta-analysis, (4) single gene polymorphism was considered, (5) stratified analysis by other related susceptible factors, such as diet, infection, smoking, alcohol etc. have not been conducted due to unavailability of sufficient data, and (6) gene–environment interactions were not considered.

In conclusion, the results of present meta-analysis suggested that MTHFR C677T polymorphism is a risk factor for male infertility in Asian population [T vs. C: OR = 1.99 (1.58–2.51)]. Future well designed large studies with more studies and larger sample size might be necessary to validate this association in different populations including other risk factors in the susceptibility of male infertility.

Acknowledgement The author is highly grateful to Leon Bax (Chief Scientific Officer at BiostatXL, UMC Utrecht) for his valuable suggestions, which help in statistical analysis.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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