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RESEARCH PAPER

An in-depth study of specific pathway associated to abnormal reproductive system: A meta-analysis in a specific Caucasian ethnicity

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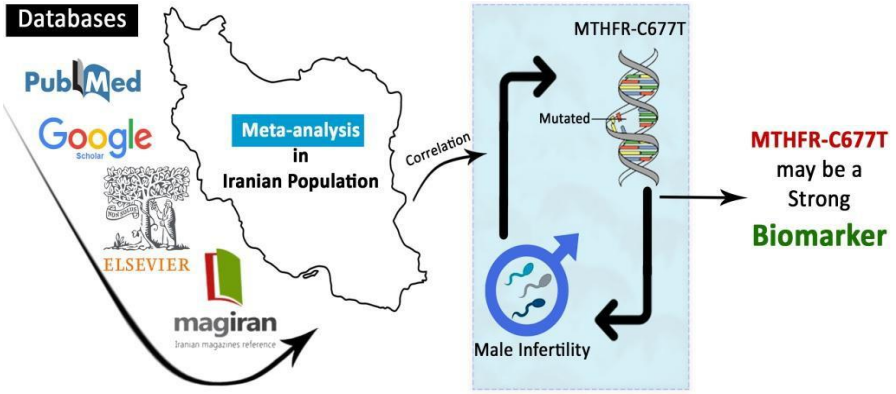
Highlights

- The methylene tetrahydrofolate reductase gene has a chief role in DNA truthfulness.
- The rs1801133 is a common variation in MTHFR gene.
- The C677T gene variation is associated with the risk of male infertility.

Graphical Abstract



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Abstract

Human methylenetetrahydrofolate reductase (MTHFR) gene plays a vital role in folate metabolism. This gene is located on chromosome 1 and has 12 exons. Many single nucleotide polymorphisms are found in MTHFR gene, in which the C677T variation is one of the key polymorphism. In this literature review, four experimental studies investigating the correlation of the abovementioned polymorphism with male infertility in Iranian population were enrolled (a specific Caucasian ethnicity). To obtain more comprehensive outcomes, a meta-analysis was made by data extraction from four published papers. Eligible studies were found through a comprehensive search using appropriate electronic databases and were analyzed by Metagenyo online software. Finally, significant associations between C677T gene polymorphism and male infertility in TT vs. CC, CT vs. CC, TT+TC vs. CC, and TT vs. TC+CC genetic models were found in Iranian population with no publication bias. MTHFR gene polymorphisms could be associated with infertility in men. Thus, determination of a polymorphism genotype in this gene could be useful for infertile individuals screening.

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Introduction

Infertility occurs in 15% of couples. Half of the causes of infertility are related to male factors. Furthermore, about 15-30% of these male factors are related to genetic factors (1). Defects in genes involved in spermatogenesis can cause infertility (2). Polymorphisms in autosomal genes such as CFTR, and BRCA2, have been shown to play an essential role in male infertility (3).

Another gene in which polymorphisms can increase the risk of infertility is the MTHFR gene. The product of this gene is involved in the conversion of homocysteine to methionine (4). Deficiency in this gene causes homocysteine accumulation and folate depletion, leading to hyperhomocysteinemia and decreased serum folate. Studies show that folate deficiency increases DNA failure (5). Hyperhomocysteinemia can be caused by low MTHFR gene activity or a deficiency in vitamin B12 uptake (6). Low levels of vitamin B12 and folate in serum are responsible for two-thirds of homocysteinemia cases (7). The amount of vitamin B12 that a person absorbs during the day determines a person's serum homocysteine level. High serum homocysteine levels can be affected by environmental factors such as smoking, heavy coffee consumption, and low physical activity (8).

Single nucleotide polymorphisms (SNPs) are abundant in the MTHFR gene, but there are three common SNPs C677T (rs1801133), A1298C (rs1801131), and G1793A (rs2274976) in this gene. C677T is more common than the other two (9). Polymorphisms in this area are associated with many diseases such as cardiovascular abnormalities, depression, and a wide range of cancers (10). The frequency of the 677T allele varies significantly in different populations. The allele frequency varies from 30% to 40% in Europe and the United States to 5% to 10% in Africa and Sri Lanka. Previous studies have shown that individuals with the 677TT genotype have more deficits in intracellular methylation and higher serum homocysteine (6, 11).

These four studies regarding the correlation of C677T gene transition with male infertility in the Iranian men population, but the outcomes are questionable. Thus, the relationship between this polymorphism and male infertility in the Iranian population was investigated by meta-analysis with a creative approach.

Materials and Methods

Search procedure

Some suitable global databases were used, such as Google Scholar, PubMed, Web of Science, and Persian records such as Scientific Information (SID) and Magiran, to discover eligible articles. Our electronic search keywords were as follows: polymorphism, MTHFR gene, methylenetetrahydrofolate reductase, male infertility, Iran, Iranian population, C677T, SNP, and mutation.

Study collection and information extraction

The numerous papers in the initial research were checked, and the citations of all reached papers were assessed. The following criteria were considered for inclusion of article in meta-analysis: (1) Papers on human; (2) studies investigating the correlation of C677T variation with male infertility; (3) case-control studies; (4) adequate data for computing the odds ratio (OR), and their 95% confidence interval (CI). However, the exclusion criteria were as follows: (1) without sufficient data for the estimating of ORs and 95% CIs; (2) Studies with other ethnicities except for the Iranian race.

Quantitative synthesis

A Chi-square test was employed to evaluate the Hardy-Weinberg equilibrium (HWE) in all included studies in our meta-analysis. ORs and 95% CI measured the strength of correlation between MTHFR-C677T gene variation and risk of male infertility.

The quantitative synthesis was performed for the four following models: Co-dominant homozygote (TT vs. CC), Co-dominant heterozygote (CT vs. CC), dominant (TT+TC vs. CC), and recessive (TT vs. TC+CC) models. The heterogeneity among studies was estimated by I^2 score and Q test (12), and when the p-value of the heterogeneity was >0.1, the fixed-effect model was employed (13). Otherwise, the random-effect model was

used (14). The publication bias was evaluated by Egger's test (15) and Begg's funnel plots (16). The Metagenyo online software was used for the statistical analysis.

Results

The search strategy and study selection procedure based on PRISMA criteria is shown in Figure 1. After screening possible suitable papers, it is concluded on four eligible articles (10, 17, 18). Some features of these four eligible studies, including authors' names, publication date, genotyping method, genotype frequencies, are detailed in Table 1. The frequencies of genotypes in control groups of all four studies met Hardy-Weinberg criteria (19, 20).

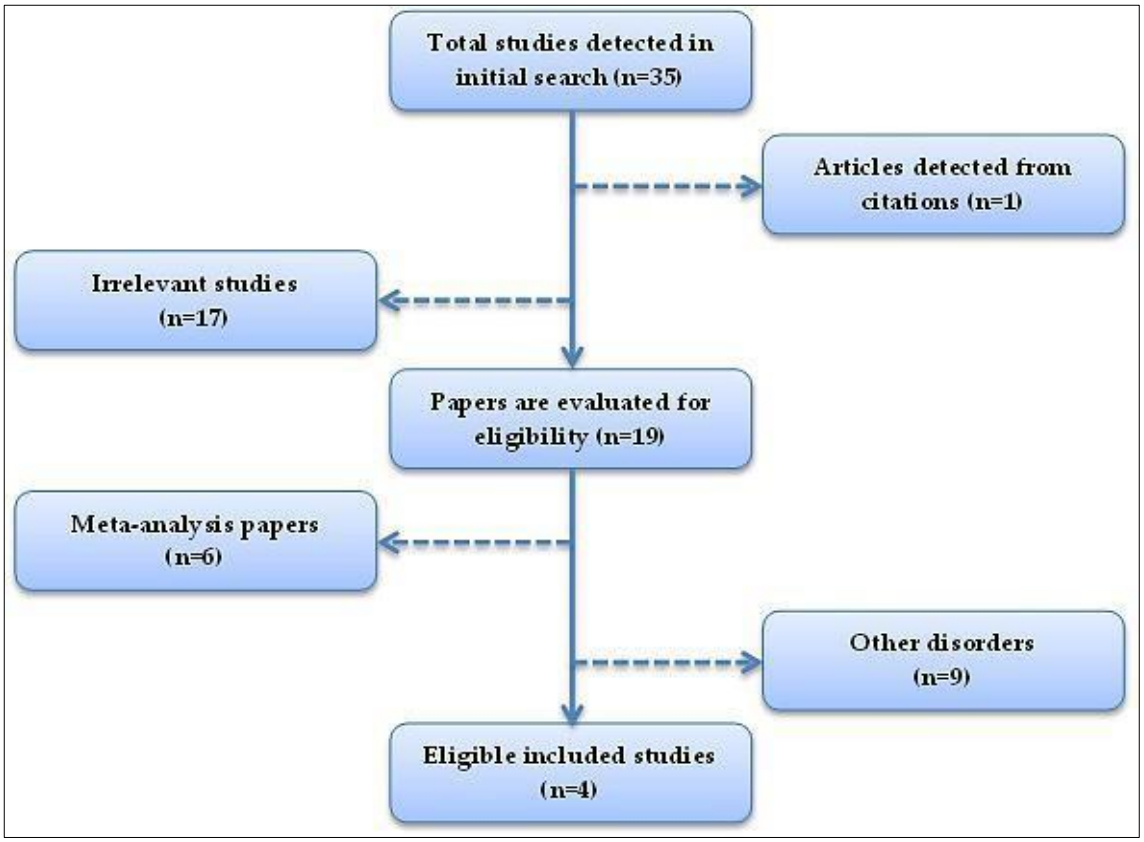

Figure 1. Flowchart for search strategy.

Table 1. Features of included studies in the meta-analysis.

Study	P_{HWE}	Adjusted $_{HWE}$	Genotyping method	TT Cases	TC Cases	CC Cases	TT Controls	TC Controls	CC Controls
Safarinejad et al., (21)	0.83	0.826	PCR-RFLP	26	80	58	36	148	144
Nikzad et al., (19)	0.48	0.720	PCR-RFLP	24	109	109	13	98	144
Karimian and Colagar, (13)	0.09	0.261	PCR-RFLP	8	59	51	3	52	77
Najafipour et al., (18)	0.31	0.619	Sequencing	44	123	113	11	43	66

PCR: polymerase chain reaction; RFLP: restriction fragment length polymorphism. PHWE: The p-value related to Hardy-Weinberg equilibrium in the control group.

Table 2. Results of the meta-analysis.

Genetic model	Analysis Model	OR (95%CI)	*P*-value	tau²	Q (df=3)	*P*H	I²
Codominant model (TT *vs*. CC)	Random effect	2.2136 (1.5262; 3.2106)	< 0.001	0.000	1.302	0.7266	0%
	Fixed effect	2.2136 (1.5262; 3.2106)	< 0.001	-	1.302	0.7266	0%
Codominant model (CT *vs*. CC)	Random effect	1.5132 (1.2215; 1.8745)	< 0.001	0.000	0.7568	0.8598	0%
	Fixed effect	1.5132 (1.2215; 1.8745)	< 0.001	-	0.7568	0.8598	0%
Dominant model (TT+TC *vs*. CC)	Random effect	1.6249 (1.3247; 1.9931)	< 0.001	0.000	0.9022	0.8249	0%
	Fixed effect	1.6249 (1.3247; 1.9931)	< 0.001	-	0.9022	0.8249	0%
Recessive model (TT *vs*. TC+CC)	Random effect	1.8151 (1.2753; 2.5835)	< 0.001	0.000	1.126	0.7708	0%
	Fixed effect	1.8151 (1.2753; 2.5835)	< 0.001	-	1.126	0.7708	0%

OR, odds ratio; CI, confidence interval; *P*H, *P*-values for heterogeneity from Q test.

The association results of the meta-analysis are summarized in Table 2. As revealed in this table, a true association between studied gene variation was observed with male infertility in the Co-dominant model (TT vs. CC), Co-dominant model (CT vs. CC), Dominant model (TT+TC vs. CC), Recessive model (TT vs. TC+CC) genetic models. Also, there were not any significant heterogeneities among the included studies. Also, publication bias evaluation revealed that there are no significant publication biases in our meta-analysis. The data from publication bias is showed in Figure 2. The Egger's test was estimated for TT vs. CC as p-value= 0.0637, for CT vs. CC as p-value= 0.2444, for TT+TC vs. CC as 0.278, and for TT vs. TC+CC as0.057.

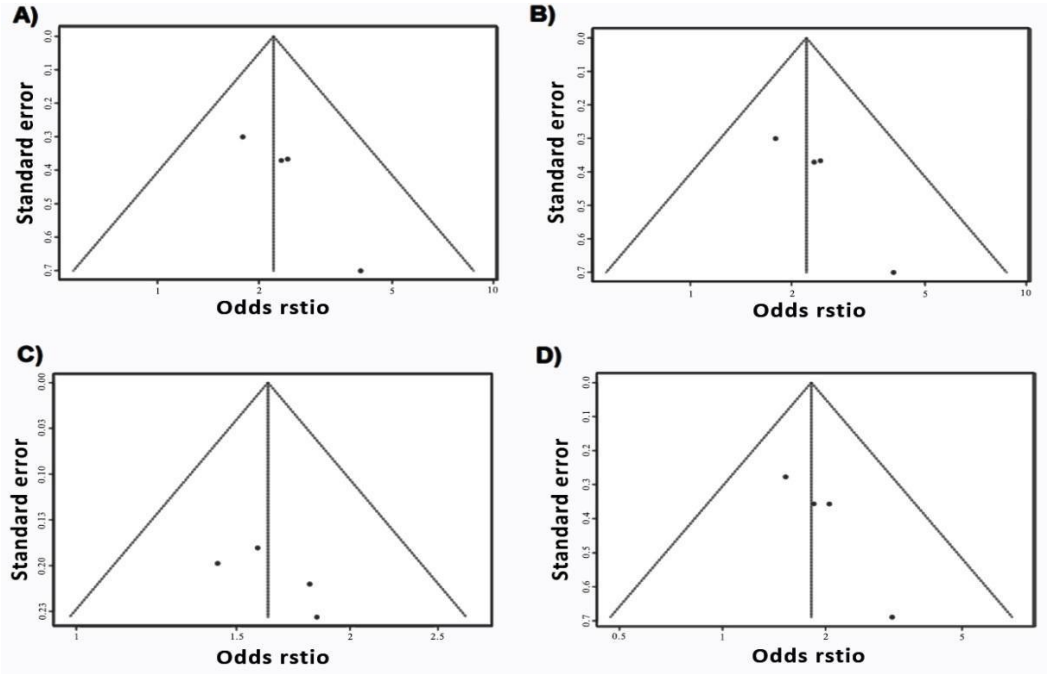

Figure 2. Funnel plot. Results of meta-analysis in TT vs. CC (A), CT vs. CC (B), TT+TC vs. CC (C), and TT vs. TC+CC (D) models.

Discussion

In the present study, the association of MTHFR-C677T gene mutation has been evaluated with male infertility risk in the Iranian population by a meta-analysis. The obtained data revealed significant associations between the mentioned polymorphism and male infertility in four heterozygotes, homozygote, dominant, and recessive genetic models. These results could be strong evidence stating the biomarker feature of this variation. On the other hand, there was no significant publication bias in the outcomes. So this data could be a reason why the obtained meta-analysis is reliable.

While the relation between polymorphisms in the MTHFR gene and infertility in men is unclear, several mechanisms can explain the cause of infertility due to mutations in the MTHFR. First, spermatogenesis is a complex process that involves several genes, and methylation of these genes has a significant effect on the expression of these genes (20). DNA hypomethylation causes an error in the differentiation of germ cells into spermatocytes (21). Induction of hypomethylation in mice by treatment with 5-aza-deoxycytidine prevents spermatogonia from differentiating into spermatocytes (21). Second, mutations in the MTHFR gene cause auto-oxidation and the production of reactive oxygen species (ROS) (22, 23). Increased ROS production increases DNA damage mediated by increased homocysteine (24). Human sperm susceptible to oxidative stress, and antioxidants such as folate can relieve oxidative stress and protect sperm DNA against damage. In humans, sperm produce ROS, leading to high spermatozoon activity, acrosome reaction, and sperm attachment to oocytes (25, 26). Increased peroxidation of membrane lipids leads to changes in membrane fluidity, so sperm function is impaired due to metabolic defects, defects in acrosome activation, and the inability of sperm to fuse with oocytes. Increased membrane lipid peroxidation ultimately leads to a decrease in sperm count, sperm motility, and loss of sperm morphology (27). Thus, increased DNA damage due to increased homocysteine by oxidative stress can be infertility in the MTHFR gene (28). Genetic variations may change the RNA structure, gene expression, and function of the protein. A previous study showed that MTHFR-C677T genetic polymorphism could impact protein function and structure of RNA (18).

Conclusion

Based on results, the MTHFR-C677T mutation could be considered a genetic risk factor male infertility in Iran's genetic risk factor for male infertility in Iran. However, there are some limitations in our study which should be mentioned. For example, the number of eligible studies included in the meta-analysis was too low. Therefore, more studies with larger sample sizes are essential to finding more precise outcomes. Moreover, further studies regarding gene-gene and gene-environmental factors such as folate intake could help reach more accurate outcomes.

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