

## RESEARCH PAPER

# Study of A1298C variation in MTHFR gene as a molecular risk factor for male infertility in Iran

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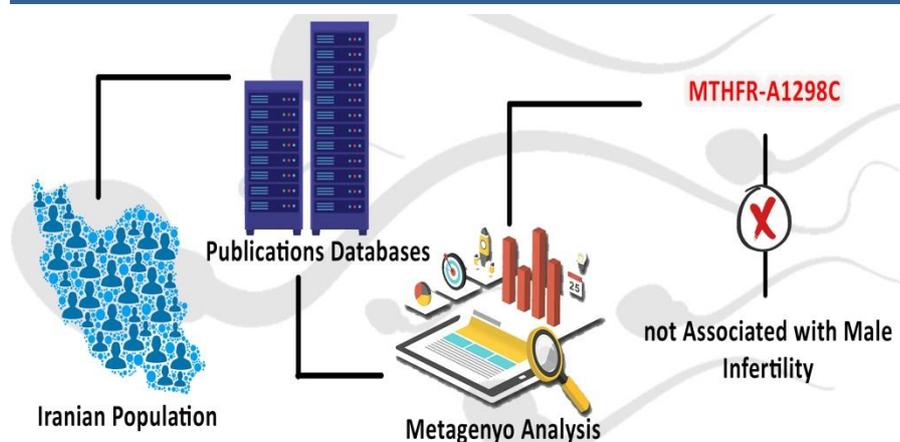
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## Highlights

- Methylene tetrahydrofolate reductase molecule could be a key enzyme in cellular processes.
- The A1298C genetic variation is an exonic SNP in MTHFR sequence.
- The A1298C genetic polymorphism is not correlated with male infertility risk.

## Graphical Abstract



## Article Info

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## Abstract

Methylenetetrahydrofolate reductase is an important enzyme in folate metabolism pathway. Some studies investigated the correlation of a common gene variation (A1298C) with male infertility in a specific Caucasian population (Iran), but the outcomes are controversial. Thus, the current study employed a meta-analysis to determine the association of mentioned variation with male infertility in Iranian population. PubMed, Google Scholar, EMBASE, SID, and Magiran databases were searched to recognize eligible publications. The odds ratios (ORs) was used with corresponding 95% confidence intervals (95% CIs) to assess the strength of genetic association analysis. The data were analyzed through Metagenyo software. Generally, three qualified papers were found and included in the quantitative synthesis (meta-analysis). The obtained data displayed that the variation is not correlated with male infertility in co-dominant genetic models. Also, the results showed no significant heterogeneities among eligible included studies. Moreover, publication bias was not detected in the current analysis. A1298C polymorphism in MTHFR gene is not correlated with male infertility in Iranian population, and it could not be considered as a risk factor for male infertility.

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## Introduction

Infertility is the incapability to attain gestation after one year of unprotected sexual intercourse and could involve about 15% of couples (1). About 50% of infertility reasons are related to male aspects. Some male infertility factors are considered anatomical aberrations such as ductal obstructions, varicoceles, or ejaculatory disorders (2). However, some other factors of male infertility are related to genetic factors (3). Impairments in genes and proteins involved in the production of sperm can be a source of infertility in men (4). Genes that are considered as risk factors for spermatogenesis include genes encoding protamines (5), telomere processing genes (6), genes involved in detoxification (7), and numerous other genes. However, an important group of these genes, which are considered serious spermatogenesis options, are folate metabolizing genes (8).

There are three significant genes in the folate pathway: methylenetetrahydrofolate reductase (*MTHFR*), methionine synthase (*MS*), and methionine synthase reductase (*MTRR*). These three enzymes play a crucial role in DNA methylation and synthesis (8). *MTHFR* reductase is one of the main enzymes in DNA synthesis and re-methylation (9). The *MTHFR* gene converts 5, 10 methyl THF to 5-methyl THF. Methionine synthase catalyzes the transfer of a methyl group from 5-methyl THF to homocysteine, resulting in methionine production. In this pathway, 5, 10 methyl THF is involved in DNA synthesis by converting dUTP to dTTP (10). However, the *MTRR* enzyme is involved in converting methionine to homocysteine, so it is a methylation agent (11).

The A1298C genetic variation is an exonic polymorphism positioned on the eighth exon of the *MTHFR* gene, and it might influence the synthesis of DNA methylation of DNA and repair of DNA (12). Many papers are studying the correlation of A1298C polymorphism with male infertility worldwide (13). However, some limited studies are evaluating the correlation of the mentioned gene variation with the risk of male infertility in Iranians (14). This study aimed to evaluate the association of rs1801131 gene polymorphism with male infertility in the Iranian population, based on an approach of systematic review and meta-analysis (15).

## Materials and Methods

### *Search strategy, inclusion criteria, and data extraction*

Related papers were recognized by conducting a regular search in Google Scholar, PubMed, EMBASE, SID, and Magiran datasets by the following keywords: *MTHFR*, variation or single nucleotide polymorphism or SNP or mutation or A1298C, and male infertility and Iran. The electronic search was updated on October 10, 2020. Also, the citations of the recognized papers and reviews were screened to discover possible additional suitable publications.

The following inclusion principles were considered for study choice: 1) studies evaluating the correlation of *MTHFR*-A1298C variation and male infertility 2) case-control studies, and 3) adequate genotype information was reported to calculate the odds ratio (OR) and 95% confidence interval (CI). Exclusion principles were as follows: 1) reviews; meta-analysis, case reports 2) duplicate papers, and 3) inadequate information for OR and 95%CI calculation.

Two colleagues dependently studied the papers and extracted the following data from each qualified study: the author name, date of publication, number of controls and cases; genotype and allele frequency, the status of Hardy-Weinberg equilibrium (HWE) in control groups; and method of genotyping.

### *Statistical analysis*

The test of Chi square assessed the HWE. The strength of the correlation between *MTHFR*-A1298C gene variation and the male infertility risk was calculated by OR with 95% CI in two genetic models, including homozygote co-dominant (CC vs. AA) and heterozygote co-dominant (AC vs. AA) genetic models. A statistical way to define heterogeneity among the included studies was done using *Q*-test and *I*<sup>2</sup> score (16). In the mentioned test, a P-value of more than 0.10 shows the lack of heterogeneity, and then the pooled ORs were assessed using the fixed-effect model (17). Otherwise, a model of random-effect was used to calculate ORs (18).

Possible publication bias was qualitatively diagnosed using Begger's funnel plots, and Egger's test was done to detect the funnel plot's asymmetry (19, 20).

**Results**

*Characteristics of eligible included studies*

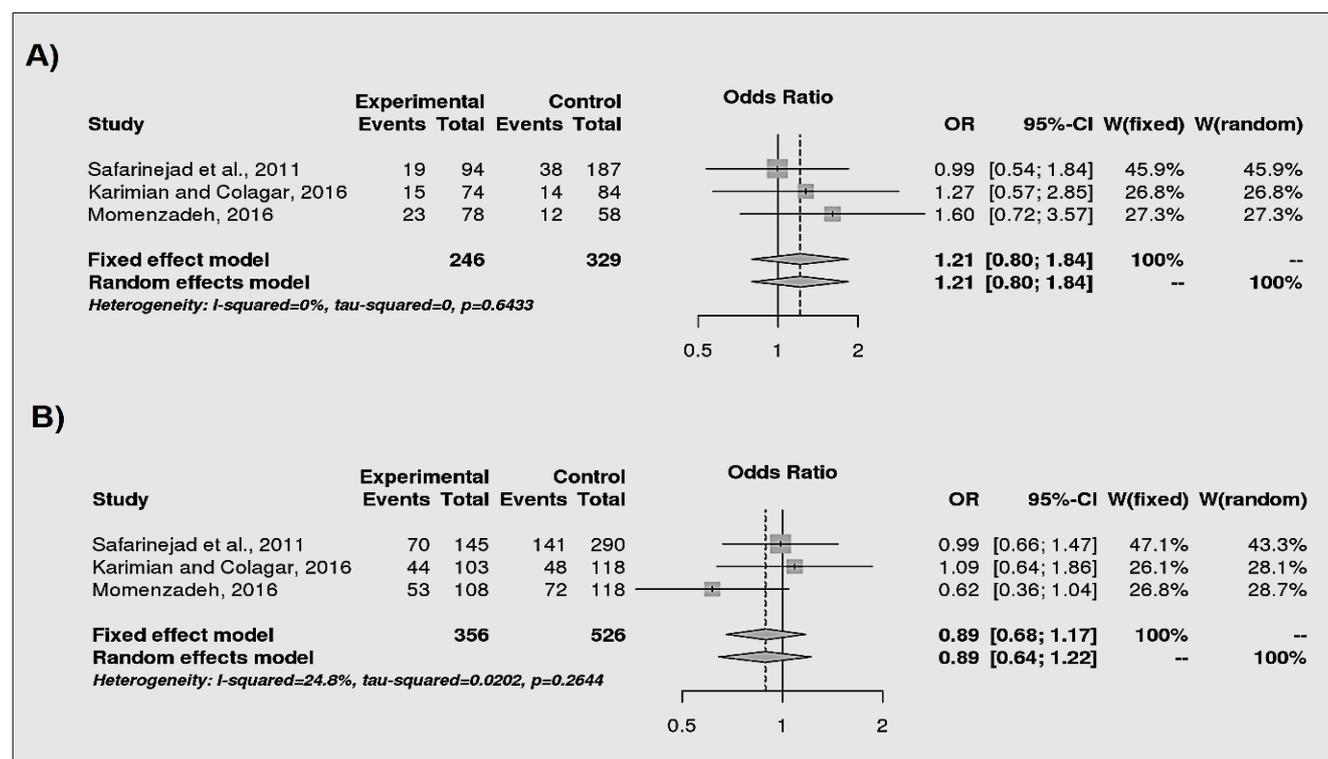
The extracted data from included studies in a meta-analysis are detailed in Table 1. , The distribution of defined genotypes frequencies in all of studies has not deviated from the Hardy-Weinberg equilibrium in control groups. All three included studies were used the PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) method for A1298C polymorphism genotyping (12). The control groups were in Hardy Weinberg equilibrium.

**Table 1.** Distribution of A1298C in included studies.

Genotype frequencies						HWE <i>P</i> <sup>a</sup>	Genotyping method	Reference
Control			Case					
AA	AC	CC	AA	AC	CC			
149	141	38	75	70	19	0.60	PCR-RFLP	Safarinejad et al., (12)
70	48	14	59	44	15	0.29	PCR-RFLP	Karimian and Colagar, (10)
46	72	12	55	53	23	0.09	PCR-RFLP	Floris et al., (11)

*Association analysis of rs1801131 with male infertility*

The association and heterogeneities results are summarized in tables 2-5. As mentioned above, there was no any deviation from HWE in control groups of all three included studies ( $P_{HWE} > 0.05$ ). The quantitative synthesis regarding the correlation of A1298C SNP with male infertility displayed that there is no substantial correlation between the mentioned variation and risk of male infertility in both CC vs. AA (OR= 1.209, 95%CI= 0.80-1.84,  $p= 0.373$ ) and AC vs. AA (OR= 0.892, 95%CI= 0.68-1.17,  $p= 0.411$ ) genetic models. Moreover, heterogeneity examination displayed no substantial heterogeneity in both CC vs. AA ( $I^2= 0\%$ ,  $P_{heterogeneity}= 0.643$ ) and AC vs. AA ( $I^2= 24.83\%$ ,  $P_{heterogeneity}= 0.264$ ) genetic models (Figure 1).



**Figure 1.** Forest plot. Results of meta-analysis in CC vs. AA (A) and AC vs. AA (B) models.

**Table 2.** Association results for CC vs. AA model.

Model	OR	95%-CI	P-value	Adjusted p-value
Fixed effect	1.2092	[0.7962; 1.8363]	0.3729175355	1
Random effect	1.2092	[0.7962; 1.8363]	0.3729175355	1

**Table 3.** Heterogeneity tests for CC vs. AA model.

tau <sup>2</sup>	H	I <sup>2</sup>	Q	P-value
0	1	0	0.8821	0.6433

**Table 4.** Association test results for AC vs. AA model.

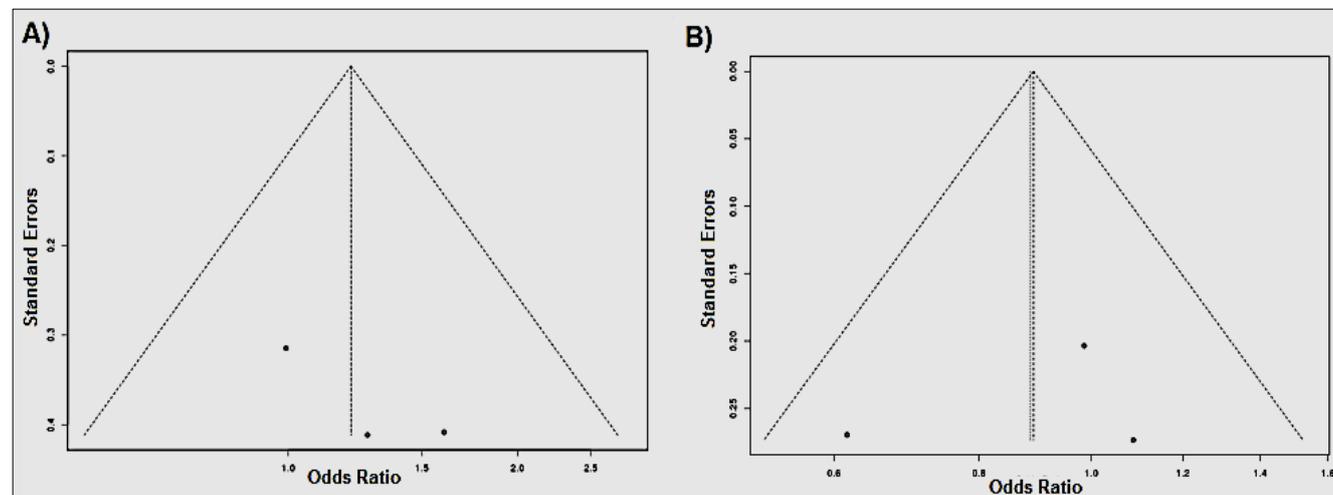
Model	OR	95%-CI	P-value	Adjusted p-value
Fixed effect	0.8916	[0.6781; 1.1723]	0.4111279161	1
Random effect	0.8855	[0.6430; 1.2194]	0.4563002267	1

**Table 5.** Heterogeneity tests for AC vs. AA model.

tau <sup>2</sup>	H	I <sup>2</sup>	Q	P-value
0.0202	1.1534	0.2483	2.6607	0.2644

### Publication bias analysis

Also, the publication bias assessment showed no significant publication biases in the present meta-analysis. The data from publication bias as funnel plots has been shown in [Figure 2](#). The Egger's test was assessed for CC vs. AA as p-value= 0.296, while this was evaluated as p-value= 0.752 for AC vs. AA genetic model.

**Figure 2.** Funnel plot. Results of meta-analysis in CC vs. AA (A) and AC vs. AA (B) models.

### Discussion

The folate pathway contains many key genes that the genetic variations in these genes may alter the male infertility risk. In the present project, the correlation of MTHFR-A1298C gene variation with male infertility in Iranian men was evaluated using a quantitative synthesis approach. In this analysis, three eligible papers were included (12). After analysis, it is found that there are no true correlations between the above-mentioned SNP and male infertility in both genetic models within Iranian men. Therefore, the A1298C gene variation may not be counted as a genetic risk factor for male infertility in Iranian population (14). Heterogeneity analysis also showed no true heterogeneities among included studies. Publication bias also showed no publication bias in current meta-analysis. Therefore, the data could be reliable and robust (15).

Folate and its cofactors, such as vitamins B2, B6, and B12, maintain intracellular nucleotides balance. Impaired folate levels can replace uracil with thymine in the DNA structure, leading to point mutations in DNA (21). The presence of uracil in the genome disrupts the DNA repair process, resulting in chromosome breakdown. Incomplete methylation resulting from disruption of the folate cycle can activate inactive transposons, resulting in genome instability (22). The DNA stability of sperm and its chromosome structure of it in fertilization process were well identified. Infertile men often have broken DNA (23).

On the other hand, the genome in the sperm head is highly compressed. This compression results from the formation of disulfide bonds between the protamine's oxidized cysteine residues (24). After fertilization, the sperm nucleus loses its compactness and forms a pre-nucleus. Low levels of free cysteine cause protamine disulfide bonds, resulting in loss of sperm nucleus compression. Thus, folate and homocysteine levels play a vital role in preserving sperm DNA (25).

Homocysteine is an amino acid containing sulfur. The metabolism of homocysteine, folate, and methyl groups is an interrelated process (26). The impaired function of enzymes in the folate cycle causes homocysteine to accumulate. Accumulation of homocysteine leads to the induction of inflammatory cytokines, which cause abnormal sperm parameters (27). Homocysteine also reduces nitric oxide (NO). Nitric oxide is an endothelium-dependent vasodilator produced by the endothelial isoform of nitric oxide synthase (28). The NO signaling pathway plays a crucial role in sperm motility, acrosome response, and fertilization (29). Thus, disruption of the folate pathway causes infertility in men in various ways (30). Therefore, it should not be overlooked that polymorphisms in genes involved in folate metabolism, including the MTHFR gene, are associated with altered infertility risk (31).

## Conclusion

Folate has vital roles in the synthesis of DNA, genome methylation, and synthesis of protein. Folate shortage could damage the action of the mentioned procedures and lead to the accumulation of homocysteine, resulting in extreme oxidative stress. This sequence of procedures is intricate in some disorders, such as failure in male fertility. Methylenetetrahydrofolate reductase and some other key enzymes have the main role in homocysteine pathways and folate metabolism. The studied variation may not be a molecular risk factor for male infertility in the Iranian population. However, this study's main limitation was the limited number of studies included in the quantitative synthesis. Then, more experimental studies are essential to gain more truthful outcomes. Moreover, in vivo and in vitro studies could be helpful for providing molecular evidence.

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