Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1746-1755 | ISSN:2251-6727

# Genetic Insights into Male Infertility: Role of MTHFR 1298 and 677 Variants

A. Sesho Narendra*, K. Geetha Kumari, G. Sudhakar

*Department of Human Genetics, Andhra University, Visakhapatnam, Andhra Pradesh, India.*

***(Received: 04 February 2024 Revised: 11 March 2024 Accepted: 08 April 2024)***

**KEYWORDS**
Male infertility, Sperm DNA fragmentation, MTHFR gene variants, Semen analysis, Gene-gene interactions

**ABSTRACT:**

**Introduction:** Male infertility, often characterized by abnormalities in sperm quantity, motility, or morphology, presents a significant challenge in reproductive health. Understanding its causes and mechanisms is crucial for developing effective treatments.

**Objectives:** To identify nucleotide polymorphisms associated with male infertility, focusing on variants 1298 A>C and 677C>T in the MTHFR gene.

**Methods:** We enrolled 243 male infertility patients from Visakhapatnam district, Andhra Pradesh, India. Participants aged 25 to 50 had tried unsuccessfully to become pregnant for at least a year. Semen samples were analyzed for physical characteristics and DNA fragmentation using the Sperm Chromatin Dispersion (SCD) assay. Single nucleotide polymorphisms (SNPs) associated with infertility were analyzed using ARMS-PCR. SPSS, Epi Info and MDR software were used for statistical analysis.

**Results:** The analysis showed high levels of sperm DNA fragmentation, with 65% of participants showing significant DNA damage. ARMS-PCR identified MTHFR variants associated with male infertility. Genotype frequencies varied significantly, with chi-square tests indicating associations between specific genotypes and infertility risk. The odds ratios indicate an increased probability of infertility with certain SNP variants. MDR analysis revealed complex gene-gene interactions that contribute to infertility.

**Conclusions:** Our study highlights sperm DNA damage prevalence and significant genetic variants associated with male infertility in Andhra Pradesh, India. Significant associations were found with MTHFR 1298A>C polymorphism, suggesting the importance of genetic factors in male infertility and potential targets for diagnostic and therapeutic interventions.

## 1. Introduction

Male infertility is a complex condition affecting around one-third of infertility cases globally [1]. It is characterized by defects in sperm number or quality, with genetic factors playing a significant role. In India, nearly 50% of infertility cases are attributed to male reproductive anomalies [2]. The genetic landscape of male infertility is intricate, involving over 2,000 genes in spermatogenesis [3]. Semen abnormalities like azoospermia, oligoasthenoteratozoospermia are common, with male infertility being poorly responsive to primary treatments [4]. The incidence of infertility worldwide is 1 in every 7 couples, with male factors contributing to 50% of cases [5]. Understanding the genetic basis of male infertility is crucial for effective management and counseling, as approximately 40% of cases have unknown genetic etiology.

The molecular function of the 5,10-Methylenetetrahydrofolate reductase (MTHFR) enzyme in humans involves playing a crucial role in folate and homocysteine metabolism, specifically in catalyzing the conversion of 5, 10-methylene tetrahydrofolate to 5-methyl tetrahydrofolate. This conversion is essential as 5-methyltetrahydrofolate is the primary circulatory form of folate utilized in homocysteine remethylation to methionine [6]. Additionally, MTHFR is involved in DNA synthesis, DNA methylation, and acts as a catalyst in the folate metabolism pathway, crucial for gene regulation and DNA methylation processes [7].

Polymorphisms are variations in genes that can impact enzyme activity and function. In the case of the Methylenetetrahydrofolate reductase (MTHFR) gene, polymorphisms like C677T and A1298C have been associated with different health outcomes [8][9][10].

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1746-1755 | ISSN:2251-6727

The prevalence and impact of the 677C>T and 1298A>C variants of the MTHFR gene vary across different health conditions. The 677C>T variant is associated with increased risk of bladder cancer in Asians but not in Caucasians [11][12]. On the other hand, the 1298A>C variant shows significant associations with attention-deficit hyperactivity disorder (ADHD), bipolar disorder (BD), and schizophrenia (SCZ). Additionally, in breast cancer patients from the Pakistani population, the 1298A/C polymorphism exhibits a protective effect against breast cancer, particularly in homozygous C genotypes [13].

Understanding how MTHFR polymorphism impacts male reproductive health is crucial due to its association with male infertility [14]. MTHFR gene variants like C677T and A1298C have been linked to increased male infertility risk, affecting spermatogenesis and oocyte maturation, leading to cytologic anomalies. These polymorphisms can result in reduced enzyme activity, elevated homocysteine levels, and detrimental effects on sperm production [15]. Understanding these genetic variations is essential for fertility treatments, especially in cases of severe and long-standing infertility, as they can impact the success of assisted reproductive technologies like IVF/ICSI [16]. Moreover, counseling individuals with MTHFR polymorphisms about potential risks and implications for their own health and that of future offspring is crucial for informed decision-making.

In summary, the investigation of genetic polymorphisms within MTHFR genes provide insights into their involvement in male infertility. Understanding these genetic factors and their impact on sperm production, quality, and hormonal regulation can inform targeted interventions and treatment strategies to address male infertility effectively.

## 2. Objectives

The aim of this study was to investigate various aspects of male infertility through comprehensive analysis. The objectives of this research were to analyze sperm parameters, evaluate sperm DNA fragmentation and its effects, and perform genetic analysis to identify polymorphisms associated with male infertility. The study identified nucleotide polymorphisms in infertile men, focusing on MTHFR 1298A>C and 677C>T variants. In addition, the study analyzed the association between identified polymorphisms and male infertility, including their correlation with abnormal semen parameters.

## Methods

### Study Population:

Using the formula proposed in [17], a total of 243 male infertility patients aged 20-50 years were recruited from the Vasudha Women & Children Hospital, Medicover Hospital and Sri Clinic, the infertility clinics in Visakhapatnam district, Andhra Pradesh, India.

Men engaged in regular unprotected sexual activity for at least a year without success were considered eligible for the study. Men with various semen abnormalities ranging from mild to severe were included. However, men who exhibited azoospermia or had particular genetic infertility causes, history of cancer, cryptorchidism, vasectomy, and if their partners had polycystic ovarian syndrome, endometriosis, or salpingitis were excluded from consideration.

### Semen Sample Collection and Analysis:

After abstaining for 48 hours to 6 days, semen samples were taken. Carefully documented, the collection was carried out in separate rooms next to the laboratory. The volume, pH, color, liquefaction time, and viscosity of the samples were measured. To prevent contamination, samples were gathered by masturbating into sterile containers. Incomplete samples were not examined.

### Sperm DNA Fragmentation Assessment with Sperm Chromatin Dispersion Assay (SCD):

The Sperm Chromatin Dispersion (SCD) test was employed to evaluate the extent of DNA damage in spermatozoa. This assay detects DNA damage by the presence of "halo" structures surrounding the sperm nuclei, with the size and appearance of the halos indicating the level of fragmentation. Agarose-coated slides were meticulously prepared, and semen samples were homogenized with agarose before being applied to the slides. The slides were then treated with a denaturation solution to unwind the DNA, followed by a lysis buffer to remove proteins, thereby enabling clear visualization of the DNA halos. After staining with Giemsa, the slides were examined under a microscope,

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1746-1755 | ISSN:2251-6727

where the halo size was meticulously measured to calculate the sperm DNA fragmentation index (sDFI), providing a quantitative assessment of DNA integrity.

**Analysis of Single Nucleotide Polymorphisms (SNPs) Using Probe-Based ARMS-PCR:**

Using the NucleoSpin® Tissue kit and following the manufacturer's instructions, genomic DNA was extracted from participant semen samples. After extracting DNA, Single Nucleotide Polymorphisms (SNPs) were analyzed using ARMS-PCR (Amplification Refractory Mutation System Polymerase Chain Reaction). Targeting the SNPs of interest indicated in Table 1, particular primers were designed.

The PCR master mix for each reaction was prepared by adding 5 µl 2X TB Green Premix Ex Taq together with 1 µl forward primer (1F or 2F at 10 pmol), 1 µl reverse primer (1R or 2R at 10 pmol). and 1 µl molecular water. Additionally, 2 µL of DNA sample was included in both normal and mutant reactions. PCR amplification was performed with an initial denaturation at 95°C for 3 minutes, followed by 35 cycles of denaturation at 95°C for 10 seconds each. The annealing temperature varies: it is 68°C for MTHFR 1298, and 60°C for MTHFR 677. Extension occurs at 72°C for 20 seconds in each cycle for all targets. The results of the probe-based ARMS PCR were noted through computer-generated plots or graphs. Mutant alleles were depicted in red, normal alleles in green, and heterozygous individuals, containing one mutant and one normal allele, were identified when both colors appeared.

**Table 1: Primer Sequences and Target Lengths for SNP Amplification**

| SNP | Primer Name | Primer Sequence | Target Length |
|---|---|---|---|
| **MTHFR 1298** | Forward | TTTGGGGAGCTGAAGGACTACT | 180 bp |
| | Normal Reverse | GAACGAAGACTTCAAAGACACTTT | |
| | Mutant Reverse | GAACGAAGACTTCAAAGACACTTG | 180 bp |
| **MTHFR 677** | Forward | AGTCCCTGTGGTCTCTTCATC | 220 bp |
| | Normal Reverse | AGCTGCGTGATGATGAAATCGG | |
| | Mutant Reverse | AGCTGCGTGATGATGAAATCGA | 220 bp |

**Statistical Analysis:**

Statistical analysis of the data involved the use of multiple software tools, including SPSS (v.24), Epi Info (v.5), and MDR (v.3.0.2). This involved calculating allele and genotype frequencies to understand their distribution within our study group. Chi-square tests were used to compare differences both within and between groups and to gain insight into the significance of these differences. In addition, odds ratios and corresponding 95% confidence intervals were calculated to quantify the strength of association between specific factors and outcomes of interest, thereby providing a deeper understanding of the observed relationships. In addition, we used Multifactor Dimensionality Reduction (MDR) software to examine interactions between genes to identify complex genetic patterns that contribute to infertility in our study population.

## 3. Results

The research aimed to investigate how certain gene variants correlate with DNA fragmentation in men experiencing infertility. The study focused on two significant single nucleotide polymorphisms (SNPs) within the MTHFR gene, namely 1298 A>C and 677 C>T. Analysis of the data showed diverse levels of correlation between these genotypes and DNA fragmentation, as detailed in Tables 2 and 3.

Graph 1 illustrates the distribution of MTHFR genotypes (rs1801131, A1298C, and rs1801133, C677T) among infertile men, categorized by DNA

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1746-1755 | ISSN:2251-6727

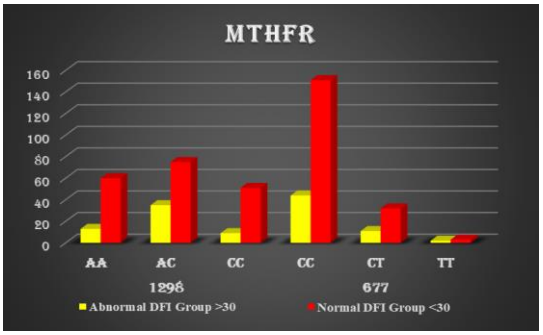
fragmentation status. In the abnormal DNA fragmentation group (>30), comprising 57 individuals, the distribution of A1298C genotypes was: 23% AA, 61% AC, and 16% CC, while for C677T genotypes, it was: 77% CC, 19% CT, and 4% TT. Conversely, in the normal DNA fragmentation group (<30), consisting of 186 individuals, the distribution for A1298C genotypes was: 32% AA, 40% AC, and 28% CC, and for C677T genotypes, it was: 81% CC, 17% CT, and 2% TT. Comparing the distributions between the two groups, we observe differences in both A1298C and C677T genotypes, indicating potential associations between MTHFR genotypes and male infertility.

For the MTHFR 1298 variant, chi-square tests were employed to compare observed and expected counts between the two groups (Table 2). In the abnormal group, the differences were not statistically significant ($\chi2=3.1250$, $p>0.05$), while in the normal group, significant differences were observed ($\chi2=6.8321$, $p<0.01$). Additionally, allele frequencies for A and C alleles were calculated, revealing discrepancies between the two groups. Intergroup heterogeneity analysis further confirmed a statistically significant difference ($\chi2=7.9700$, $p<0.01$) between abnormal and normal DNA fragmentation groups.

Similarly, for the MTHFR 677 variant, chi-square tests were used to compare observed and expected counts between abnormal and normal groups. The results indicated non-significant differences in both the abnormal ($\chi2=1.3793$, $p>0.5$) and normal groups ($\chi2=0.7170$, $p>0.5$). Allele frequencies for C and T alleles were calculated, showing no significant differences between the two groups. Intergroup heterogeneity analysis also confirmed non-significant differences ($\chi2=0.9570$, $p>0.5$) between abnormal and normal DNA fragmentation groups.

These findings underscore the distinct associations between MTHFR 1298 and MTHFR 677 variants and DNA fragmentation in abnormal and normal groups, highlighting the relevance of these genetic variants in understanding DNA fragmentation dynamics.

In the assessment of genetic associations with male infertility, Table 3 presents the results of tests for association, alongside odds ratios (OR) and 95% confidence intervals (CI) for MTHFR 1298 and 677 variants. This table summarizes the association between MTHFR gene variants (1298 and 677) and DNA fragmentation index (DFI) in both abnormal and normal groups. For MTHFR 1298, the AC genotype exhibited a significantly reduced risk of abnormal DFI (OR = 0.46, 95% CI = 0.23-0.96, p = 0.0349), while the CC genotype and combined AC+CC genotypes did not show significant associations. Allele C also did not show a significant association with abnormal DFI. For MTHFR 677, neither CT nor TT genotypes, nor their combination, showed significant associations with abnormal DFI compared to the CC genotype. Additionally, allele T did not show a significant association with abnormal DFI. These results suggest that MTHFR 1298 AC genotype may have a protective effect against abnormal DFI, while MTHFR 677 genotypes and alleles do not significantly influence DFI in male infertility.




**Graph 1: Graphical depiction of MTHFR (1298A>C and 677C>T) genotypes and allele frequencies**

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1746-1755 | ISSN:2251-6727

**Table 2: Analysis of association between MTHFR (1298A>C), (677C>T) polymorphism and risk for male infertility**

| Gene | Genotype & Alleles | DFI >30 (N=57) | | DFI <30 (N=186) | | Chi-square ($\chi^2$) | |
|---|---|---|---|---|---|---|---|
| | | Observed | Expected | Observed | Expected | DFI >30 | DFI <30 |
| MTHFR 1298 | AA | 13 | 16.32 | 60 | 51.11 | $\chi2=3.1250^{NS}$ (0.10>p>0.05) | **$\chi2= 6.8321^{**}$** (0.01>p>0.001) |
| | AC | 35 | 28.36 | 75 | 92.78 | | |
| | CC | 9 | 12.32 | 51 | 42.11 | | |
| | A | 0.5400 ± 0.0226 | | 0.5200 ± 0.0226 | | Inter Group $\chi^2$ | |
| | C | 0.4600 ± 0.0226 | | 0.4800 ± 0.0226 | | **$\chi^2= 7.9700^{**}$** (0.05>p>0.02) | |
| MTHFR 677 | CC | 44 | 42.98 | 151 | 149.94 | $\chi^2=1.3793^{NS}$ (0.50>p>0.10) | $\chi^2= 0.7170^{NS}$ (0.50>p>0.10) |
| | CT | 11 | 13.03 | 32 | 34.12 | | |
| | TT | 2 | 0.99 | 3 | 1.94 | | |
| | C | 0.8700 ± 0.0153 | | 0.9000 ± 0.0136 | | Inter Group $\chi^2$ | |
| | T | 0.1300 ± 0.0153 | | 0.1000 ± 0.0136 | | $\chi^2= 0.9570^{NS}$ (0.50>p>0.10) | |

***p<0.05, **p<0.01, ***p<0.001,** NS Non-significant, DFI – DNA Fragmentation Index

**Table 3: Test of Association, Odds Ratio, and 95% Confidence Interval Estimates of MTHFR genotypes in infertile men**

| Gene | Genotype/ Allele combinations | DFI Abnormal and Normal Groups | | |
|---|---|---|---|---|
| | | OR (95%CI) | $\chi^2$ value | p- value |
| MTHFR 1298 | AA | Reference | - | 1 |
| | AC | 0.46 (0.23-0.96) | 4.4510 | **0.0349*** |
| | CC | 1.23 (0.49-3.11) | 0.1881 | 0.6645 |
| | AC+CC | 0.62 (0.31-1.24) | 1.8540 | 0.1735 |
| | A | Reference | - | 1 |
| | C | 1.09 (0.72-1.67) | 0.1749 | 0.6758 |
| MTHFR 677 | CC | Reference | 1 | 1 |
| | CT | 0.85 (0.39-1.82) | 0.1805 | 0.6709 |
| | TT | 0.43 (0.07-2.70) | 0.8368 | 0.3603 |
| | CT+TT | 0.78 (0.38-1.61) | 0.4382 | 0.5080 |
| | C | Reference | 1 | 1 |
| | T | 0.81 (0.42-1.56) | 0.3897 | 0.5325 |

***p<0.05, **p<0.01, ***p<0.001,** NS Non-significant, DFI – DNA Fragmentation Index

**Table 4: Association of MTHFR genotypes with diagnosis (Normospermic and Abnormospermic)**

| Gene | Genotypes | Diagnosis N (%) | | OR (95%CI) | $\chi^2$ value | p-value |
|---|---|---|---|---|---|---|
| | | Ab.Spermia | Normospermia | | | |
| MTHFR 1298 | AA | 55 (22.6) | 18 (7.4) | Reference | - | 1 |
| | AC+CC | 126 (51.9) | 44 (18.1) | 1.07 (0.56-2.01) | 0.040 | 0.841 |

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1746-1755 | ISSN:2251-6727

| | | | | | | |
|---|---|---|---|---|---|---|
| **MTHFR 677** | CC | 146 (60.1) | 49 (20.2) | Reference | - | 1 |
| | CT+TT | 35 (14.4) | 13 (5.3) | 1.11 (0.54-2.26) | 0.077 | 0.781 |

Table 4 presents the association of MTHFR genotypes with the diagnosis of normospermia and abnormospermia in infertile men. For MTHFR 1298, the AA genotype was found in 22.6% of men with abnormospermia and 7.4% with normospermia, serving as the reference group. The combined AC+CC genotypes were found in 51.9% of men with abnormospermia and 18.1% with normospermia. The odds ratio (OR) for AC+CC genotypes compared to AA was 1.07 (95% CI = 0.56-2.01), with a $\chi 2$ value of 0.040 and a p-value of 0.841, indicating no significant association with the diagnosis of abnormospermia. For MTHFR 677, the CC genotype was present in 60.1% of men with abnormospermia and 20.2% with normospermia, serving as the reference group. The combined CT+TT genotypes were found in 14.4% of men with abnormospermia and 5.3% with normospermia. The OR for CT+TT genotypes compared to CC was 1.11 (95% CI = 0.54-2.26), with a $\chi 2$ value of 0.077 and a p-value of 0.781, indicating no significant association with the diagnosis of abnormospermia.

Table 5 presents results from Multifactor Dimensionality Reduction (MDR) analysis for combinations of genetic loci studied. MTHFR 1298 A>C emerged as the most effective single locus model (TBA= 0.6054, CVC=10/10, P=0.05) and MTHFR 1298A>C and 677C>T combination as the best two-locus model (TBA= 0.6156, CVC=10/10, P=0.05) for predicting infertility. These combinations, detailed in the table, highlight associations between multi-locus genotype combinations and infertility.

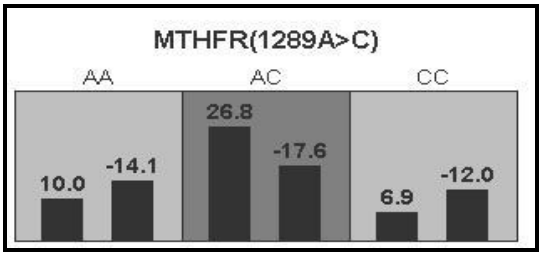
Graphs 2 and 3 depict gene combinations associated with the risk of infertility. Dark shading represents high-risk genotypes, light shading indicates low-risk and white cells signify no data. According to the single-locus model, the MTHFR (1298A>C) gene is a significant predictor of male infertility, with the AC genotype carrying the highest risk. In the two-locus models, MTHFR (1298A>C) AC genotype with CC and CT genotypes of 677C>T variant are correlated with varying levels of infertility risk. These findings provide valuable insights into the genetic factors influencing the risk of infertility.

**Gene-Gene Interactions:**

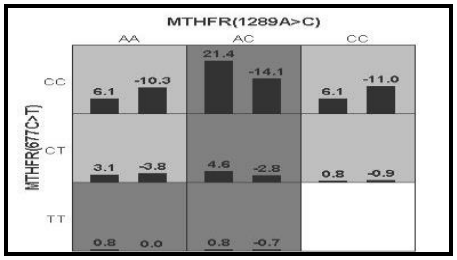
**Table 5: Multi-locus interaction model by MDR method for gene polymorphisms**

| Locus No. & Combinations | Training Bal. Acc. | Testing Bal Acc. | CVC | Sign Test (p) |
|---|---|---|---|---|
| MTHFR (1298A>C) | 0.6054 | 0.6085 | 10/10 | 8 (0.0547)* |
| MTHFR (1298A>C), MTHFR (677C>T) | 0.6156 | 0.5833 | 10/10 | 8 (0.0547)* |

***P≤0.05 based on 1000 permutations; CVC = cross-validation consistency**




**Graph 2: Single-loci model**




**Graph 3: Single-loci and Two-locus model**

High Risk  Low Risk  Empty Cell

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1746-1755 | ISSN:2251-6727

## 4. Discussion

Methylenetetrahydrofolate reductase (MTHFR) plays a crucial role in folate metabolism, essential for DNA synthesis and repair, particularly during sperm production [16]. Variations in the MTHFR gene can disrupt enzyme activity, impacting folate metabolism and increasing infertility risk, notably in diabetic males [18]. Elevated homocysteine levels, influenced by MTHFR mutations, can adversely affect spermatogenesis, underscoring the importance of assessing these single nucleotide polymorphisms (SNPs) in severe and prolonged infertility cases. Additionally, lower MTHFR activity and s-adenosylmethionine (SAM) levels in oligozoospermic men suggest a potential link between MTHFR activity, SAM concentration, and sperm quality.

Our study investigated the association between two common MTHFR gene variants, 1298 and 677, and male infertility, shedding light on their complex relationship with reproductive health. We found a significant association between the MTHFR 1298 variant and male infertility, consistent with previous research emphasizing genetic variations' impact on male reproductive health [19][20]. Studies by Gava et al. [21] and Li & Li [22] further supported these observations, elucidating molecular mechanisms underlying MTHFR gene variants and their influence on spermatogenesis. Recent works by Ledowsy et al., [23], More et al., [14], Clement et al., [15], and Pazarbasi et al., [24] reinforced the MTHFR 1298 variant's association with male infertility, contributing to a deeper understanding of genetic factors influencing reproductive health. However, some studies, such as those by Suryandari et al., [25], Gupta et al., [26], Kang [27], Ni et al. [28] and Aliakbari et al. [29] and many others did not find a significant association, adding nuance to the debate surrounding this genetic variant's role in male reproductive health.

Conversely, our results showed no significant association for the MTHFR 677 variant, contrasting with some previous studies that reported conflicting results. Bezold et al., [30], Park et al., [31] and Singh et al., [32] found an association between the MTHFR 677 variant and male infertility, suggesting a possible role of this genetic variant in male reproductive health. Additionally, Weiner et al., [33] and Huang et al., [34] emphasized the importance of folate metabolism, which was supported by the association observed in their studies. Further, Janabi et al., [35] and Barakat et al., [36] emphasized the importance of genetic variations in folate metabolic pathways, reinforcing the association between the MTHFR 677 variant and male infertility risk. However, other studies such as those by Ebisch et al., [37], Ravel et al., [38], Vani et al., [39] and Gupta et al., [40] found no significant association, which is in line with our findings. Recent studies by Balunathan et al., [41], Janabi et al., [35], Pazarbasi et al., [24] and Xiu [42] also reported no association between the MTHFR 677 variant and male infertility, contributing to the ongoing discourse.

Our study underscores a significant association between the MTHFR 1298 variant and male infertility, highlighting the potential of genetic screening for this variant in clinical practice. Future research should delve into the functional significance of MTHFR variants in male reproductive physiology and their implications for fertility management. Although our study did not find a significant association with the MTHFR 677 variant, the conflicting findings in the literature warrant further investigation into its role in male reproductive health. Discrepancies in study results may stem from various factors, including differences in sample size, ethnic composition, and methodological approaches. Additionally, the complex interplay of genetic and environmental factors in male infertility necessitates further investigation to elucidate the underlying mechanisms.

**Conclusion:**

Our study provides evidence of a significant association between the MTHFR 1298 variant and male infertility, emphasizing the potential utility of genetic screening for this variant in clinical practice. Future research should prioritize elucidating the functional significance of MTHFR variants in male reproductive physiology and their implications for fertility management. The observed discrepancies in the association between MTHFR variants and male infertility across different studies highlight the need for additional research. Specifically, more comprehensive studies are required to clarify the role of the MTHFR 677 variant in male reproductive health and its implications for fertility management. Understanding these genetic influences

could significantly enhance fertility treatments and patient outcomes.

**Acknowledgments**

The authors thank the patients, who willingly participated in the study.

**Ethical Clearance:** Ethical clearance was obtained from the Ethical Committee of Andhra University, Visakhapatnam, Andhra Pradesh, India (Approval No. IEC 54, dated 01-04-2022), and all participants provided informed consent before enrolment.

**Funding:** No funding sources

**Conflict of interest:** None declared

**References**

1. Joseph S., and Mahale S. D., 2021. Male Infertility Knowledgebase: decoding the genetic and disease landscape. Database, 2021, baab049.
2. Rajat Mahajan., Anil Kumar., 2023. Role of trans rectal and scrotal ultra-sonography in male infertility. Paripex Indian Journal of Research. 12(3).
3. Krausz C., Rosta V., Swerdloff R.S., & Wang C. 2022. Genetics of male infertility. Emery and rimoin's principles and practice of medical genetics and genomics. 121-147.
4. Agarwal A., Srivastava A., Fathima F., Lodhi B., 2023. Insight into epidemiology of male infertility in central India. International journal of reproduction, contraception, obstetrics and gynecology. 12(1).
5. Kar A., Panda S., Panda P.L., Kar T., 2017. Correlation of sperm morphology with semen parameters: a comprehensive approach to male infertility. Paripex Indian Journal Of Research. 6(5).
6. Peng J., & Wu Z., 2023. MTHFR act as a potential cancer biomarker in immune checkpoints blockades, heterogeneity, tumor microenvironment and immune infiltration. Discover Oncology. 14(1), 112.
7. Si X., Gu Q., Zhao C., Zhang X.M., Xiao T., Li Y., Ying W., & Gao S., 2022. Highly sensitive genotyping of MTHFR C677T polymorphisms using a novel RPA-LDR-qPCR assay: RPA-LDR-qPCR for MTHFR C677T genotyping. Acta Biochimica et Biophysica Sinica. 54(11), 1753.
8. Yun L., Ge M., Xu R., Zheng F., Zhao X., & Li X., 2022. C677T gene polymorphism of MTHFR is a risk factor for impaired renal function in pregnant women with Preeclampsia in the chinese Han Population. Frontiers in Cardiovascular Medicine. 9, 902346
9. Kulchenko N.G., Myandina G.I., Alhejoj H., & Tarasenko E.V., 2020. The effect of polymorphisms C677T and A1298C gene MTHFR on the reproductive function of men. Urologiia. (2), 66-70.
10. Amin Y.K., 2021. Single-nucleotide polymorphisms in MTHFR gene related to recurrent abortion. Applied Nanoscience. 1-7.
11. Saad-Hussein A., Shousha W. G., Al-Sadek S. Y. M., & Ramadan S. S., 2023. Role of MTHFR 677C> T and 1298A> C gene polymorphisms on renal toxicity caused by lead exposure in wastewater treatment plant workers. Environmental Science and Pollution Research, 30(35), 84758-84764.
12. Meng X., Zheng J. L., Sun M. L., Lai H. Y., Wang B. J., Yao J., & Wang, H., 2022. Association between MTHFR (677C> T and 1298A> C) polymorphisms and psychiatric disorder: A meta-analysis. PLoS One, 17(7), e0271170.
13. Farshid S., Alijanpour A., Barahman M., Dastgheib S. A., Narimani N., Shirinzadeh-Dastgiri Z., Maleki H., Asadian F., Mazaheri M., & Neamatzadeh H., 2022. Associations of mthfr rs1801133 (677c> t) and rs180113 (1298a> c) polymorphisms with susceptibility to bladder cancer: A systematic review and meta-analysis. Asian Pacific Journal of Cancer Prevention: APJCP, 23(5), 1465.
14. More A., Gajbe U., Olatunji O., Singh B., & Singh B.R., 2022. MTHFR Gene-Polymorphism and Infertile Men in Indian Population: A Systematic Literature Review. Cureus. 14(7).
15. Clément A., Amar E., Brami C., Clément P., Alvarez S., Jacquesson-Fournols L., and Menezo, Y. 2022. MTHFR SNPs (methyl tetrahydrofolate reductase, single nucleotide polymorphisms) C677T and A1298C prevalence and serum homocysteine levels in> 2100 hypofertile caucasian male patients. Biomolecules, 12(8), 1086.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1746-1755 | ISSN:2251-6727

16. Babu K. R., Vijayaraman M., Shastri D., & Manivannan E., 2023. Association of methylenetetrahydrofolate reductase polymorphism and male infertility in type 2 diabetes mellitus patients. BLDE University Journal of Health Sciences. 8(1), 51-55.
17. Lwanga Stephen Kaggwa., Lemeshow Stanley., World Health Organization., 1991. Sample size determination in health studies: a practical manual / S. K. Lwanga and S. Lemeshow. World Health Organization.
18. Baranizadeh K., Bahmanzadeh M., Tavilani H., Ghiasvand T., Amiri I., Yavangi M., & Shafiee G., 2022. Evaluation of methylenetetrahydrofolate reductase and S-adenosyl-methionine level in male infertility: A case-control study. International Journal of Reproductive BioMedicine. 20(4), 299-306.
19. Kumar J., Das S. K., Sharma P., Karthikeyan G., Ramakrishnan L., & Sengupta S., 2005. Homocysteine levels are associated with MTHFR A1298C polymorphism in Indian population. Journal of human genetics. 50(12), 655-663.
20. Singh K., Singh S.K., Raman R., 2010. MTHFR A1298C polymorphism and idiopathic male infertility. J Postgrad Med. 56:267–269.
21. Gava M. M., de Oliveira Chagas E., Bianco B., Christofolini D. M., Pompeo A. C. L., Glina S., & Barbosa C. P., 2011. Methylenetetrahydrofolate reductase polymorphisms are related to male infertility in Brazilian men. Genetic Testing and Molecular Biomarkers. 15(3), 153-157.
22. Li S. S., Li J., Xiao Z., Ren A. G., & Jin L., 2014. Prospective study of MTHFR genetic polymorphisms as a possible etiology of male infertility. Genet Mol Res.13(3), 6367-6374.
23. Ledowsky C., Steel A., & Schloss J., 2021. Methylenetetrahydrofolate Reductase (MTHFR) genetic polymorphisms and the risk of infertility in couples accessing Assisted Reproductive technologies: a systematic review. Advances in Integrative Medicine. 8(3), 220-229.
24. Pazarbaşı H.I., Yılmaz B., Ürünsak I.F., Keser N., Güvenmez H.K., 2023. Association of infertility and methylenetetrahydrofolate reductase genotypes in Turkish couples. Cukurova Medical Journal. 48 (1), 117-126.
25. Suryandari D.A., Wiweko B., Yunaini L., 2012. Genotype distribution of methylenetetrahydrofolate reductase A1298C and C677T gene in Indonesian infertile men. Med J Indones. 21: 23–27.
26. Gupta N., Sarkar S., David A., Gangwar P. K., Gupta R., Khanna G., Sankhwar S.N., Khanna A., & Rajender, S., 2013. Significant impact of the MTHFR polymorphisms and haplotypes on male infertility risk. PloS one. 8(7), e69180.
27. Liu, K., Zhao, R., Shen, M., Ye, J., Li, X., Huang, Y., Hua L., Wang Z., & Li, J., 2015. Role of genetic mutations in folate-related enzyme genes on Male Infertility. Scientific reports 5(1), 15548.
28. Ni, W., Li, H., Wu, A., Zhang, P., Yang, H., Yang, X., Huang X., & Jiang, L. (2015). Lack of association between genetic polymorphisms in three folate-related enzyme genes and male infertility in the Chinese population. Journal of assisted reproduction and genetics, 32, 369-374.
29. Aliakbari F., Pouresmaeili F., Eshghifar N., Zolghadr Z., & Azizi, F., 2020. Association of the MTHFR 677C> T and 1298A> C polymorphisms and male infertility risk: a meta-analysis. Reproductive Biology and Endocrinology. 18, 1-10.
30. Bezold G., Lange M., & Peter R.U., 2001. Homozygous methylenetetrahydrofolate reductase C677T mutation and male infertility. New England Journal of Medicine. 344(15), 1172-1173.
31. Park J. H., Lee H. C., Jeong Y. M., Chung T. G., Kim H. J., Kim N. K., Lee S.H., & Lee S., 2005. MTHFR C677T polymorphism associates with unexplained infertile male factors. Journal of Assisted Reproduction and Genetics. 22, 361-368.
32. Singh K., Singh S. K., Sah R., Singh I., & Raman R., 2005. Mutation C677T in the methylenetetrahydrofolate reductase gene is associated with male infertility in an Indian population 1. International journal of andrology. 28(2), 115-119.
33. Weiner A.S., Boyarskikh U.A., Voronina E.N., Tupikin A.E., Korolkova O.V., Morozov I.V., & Filipenko M.L., 2014. Polymorphisms in folate-metabolizing genes and risk of idiopathic male infertility: a study on a Russian population and a

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(3), 1746-1755 | ISSN:2251-6727

meta-analysis. Fertility and sterility. 101(1), 87-94.

34. Huang W. J., Lu X. L., Li J. T., & Zhang J. M., 2020. Effects of folic acid on oligozoospermia with MTHFR polymorphisms in term of seminal parameters, DNA fragmentation, and live birth rate: a double-blind, randomized, placebo-controlled trial. Andrology. 8(1), 110-116.
35. Al-janabi A.M., Al-Khafaji S.M., & Faris, S.A., 2022. Association of methyltetrahydrofolate reductase gene mutation, homocysteine level with semen quality of Iraqi infertile males. Egyptian Journal of Medical Human Genetics. 23(1), 51.
36. Barakat L. A., Youssef Y. E. B., Ramzy A. E., & Elsaid A. M. M., 2024. Association of the Role MTHFR 677C> T and 1298A> C polymorphisms variants as the risk factor with human male infertility. Alfarama Journal of Basic & Applied Sciences. 5(1), 90-102.
37. Ebisch I. M., van Heerde W. L., Thomas, C. M., van der Put, N., Wong W. Y., & Steegers-Theunissen R. P., 2003. C677T methylenetetrahydrofolate reductase polymorphism interferes with the effects of folic acid and zinc sulfate on sperm concentration. Fertility and sterility. 80(5), 1190-1194.
38. Ravel C., Chantot-Bastaraud S., Chalmey C., Barreiro L., Aknin-Seifer I., Pfeffer J., Berthaut I., Mathieu E.E., Mandelbaum J., Siffroi J.P., McElreavey K., & Bashamboo A., 2009. Lack of association between genetic polymorphisms in enzymes associated with folate metabolism and unexplained reduced sperm counts. PloS one. 4(8), e6540.
39. Vani G. T., Mukesh N., Rama Devi P., Usha Rani P., & Reddy P. P., 2012. Methylenetetrahydrofolate reductase C677T polymorphism is not associated with male infertility in a South Indian population. Andrologia. 44, 252-259.
40. Gupta N., Gupta S., Dama M., David A., Khanna G., Khanna A., & Rajender S., 2011. Strong association of 677 C> T substitution in the MTHFR gene with male infertility-a study on an Indian population and a meta-analysis. PloS one. 6(7), e22277.
41. Balunathan, N., Venkatesen, V., Chauhan, J., Reddy, S. N., Perumal, V., & Paul, S. F. (2021). Role of MTHFR gene polymorphisms in male infertility. International Journal of Infertility & Fetal Medicine, 12(1), 7-12.
42. Xiu Y., Cao Z., Yu D., Yang C., Wang J., Pan J., Xin Z. and Feng Y., 2024. MTHFRC 677T Gene Polymorphism and Homocysteine in North China Patients with Varicocele. Andrologia.