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Evaluation of MTHFR Gene Polymorphisms and Their Association with Recurrent Implantation Failure in Infertile Women

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Abstract

Background: Recurrent implantation failure is a distressing clinical problem in assisted reproduction and is influenced by embryonic, uterine, immunological, vascular and genetic factors. Methylenetetrahydrofolate reductase gene variants can alter folate metabolism and homocysteine regulation, creating a biologically plausible link with impaired implantation.***Objectives:*** To evaluate the distribution of MTHFR C677T and A1298C polymorphisms among infertile women with recurrent implantation failure and to assess their association with serum homocysteine levels.***Methods:*** This observational case-control study was conducted at Arundhathi Institute of Medical Sciences and Hospital, Medchal, Hyderabad, Telangana, India, from September 2025 to February 2026. A total of 100 infertile women were included, comprising 50 women with recurrent implantation failure and 50 infertile women without recurrent implantation failure. Demographic, infertility-related, biochemical and genetic data were collected. MTHFR genotypes, allele frequencies and combined risk profiles were compared between groups. ***Results:*** The groups were comparable for age, body mass index, infertility type and duration. Hyperhomocysteinemia was significantly higher in the recurrent implantation failure group than controls. MTHFR C677T CT+TT genotypes and T allele frequency were significantly higher among cases. For A1298C, the AC+CC model and C allele frequency were also significantly associated with recurrent implantation failure. High-risk combined MTHFR profiles were more frequent in cases, and hyperhomocysteinemia increased across variant genotypes.***Conclusion:*** MTHFR C677T and A1298C polymorphisms were more common among infertile women with recurrent implantation failure, with the C677T variant showing a stronger association. The findings support the relevance of folate-pathway genetic assessment and homocysteine estimation in selected women with recurrent implantation failure.

Keywords: MTHFR, C677T, A1298C, recurrent implantation failure, infertility, homocysteine, assisted reproduction.

INTRODUCTION

Recurrent implantation failure (RIF) remains one of the most challenging conditions in assisted reproductive medicine because it occurs despite repeated embryo transfer attempts and often follows extensive evaluation of ovarian, uterine and embryological factors. Recent good-practice recommendations emphasise that RIF is not a single disease entity but a clinical scenario requiring careful interpretation according to maternal age, embryo quality, number of embryos transferred and expected implantation probability [1]. Although definitions vary across studies, the condition broadly reflects failure of the embryo-endometrium dialogue during a critical period of implantation. This dialogue depends on synchronous embryonic development, endometrial receptivity, immune tolerance, vascular adaptation and molecular signalling [2,3].

The causes of RIF are heterogeneous. Embryo aneuploidy, impaired endometrial receptivity, uterine cavity abnormalities, chronic endometritis, endocrine dysfunction, thrombophilia, autoimmunity, lifestyle factors and male contributors have all been described [2-5]. Among these factors, inherited variants affecting vascular homeostasis and one-carbon metabolism are of interest because implantation requires local angiogenesis, trophoblast invasion and a balanced haemostatic environment. The methylenetetrahydrofolate reductase (MTHFR) enzyme plays a central role in folate metabolism by converting 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the methyl donor required for remethylation of homocysteine to methionine. Functional polymorphisms in the MTHFR gene, particularly C677T and A1298C, can reduce enzyme activity and contribute to raised plasma homocysteine in susceptible individuals [6,7].

Hyperhomocysteinemia has been linked with endothelial dysfunction, oxidative stress, altered placental vascular development and disturbed reproductive outcomes. The C677T variant is generally considered the stronger functional polymorphism, while A1298C has a variable effect that depends on genotype combination, folate status and population background [6-9]. Existing studies on MTHFR polymorphisms and assisted reproductive outcomes are inconsistent. Some studies and meta-analyses indicate that C677T and A1298C variants are associated with RIF or adverse IVF-related outcomes, whereas others report weak or absent associations [6,8-11]. Such differences are expected because ethnicity, sample size, folate exposure, genotyping technique, embryo selection strategy and RIF definition differ widely across studies.

Despite the controversy, evaluation of MTHFR variants remains relevant in selected infertile women with repeated implantation failure, particularly when accompanied by hyperhomocysteinemia or a thrombophilia-like clinical background. Indian data on MTHFR genotype patterns in RIF are limited, and local hospital-based evidence helps define the frequency and clinical direction of these variants. The present study was therefore conducted to evaluate the distribution of MTHFR C677T and A1298C polymorphisms among infertile women with recurrent implantation failure and infertile women without RIF, to compare allele frequencies and combined high-risk profiles, and to assess the relationship between MTHFR variant genotypes and serum homocysteine levels.

MATERIALS AND METHODS

Study design and setting

This observational case-control study was conducted at Arundhathi Institute of Medical Sciences and Hospital, Medchal, Hyderabad, Telangana, India, from September 2025 to February 2026. The manuscript was prepared in accordance with the principles of observational study reporting, and data were collected using a structured proforma designed for demographic, infertility-related, biochemical and genetic variables [14].

Study population

A total of 100 infertile women attending the infertility services were included. The study group comprised 50 women with recurrent implantation failure, defined clinically as repeated failure to achieve implantation after previous embryo transfer cycles using morphologically suitable embryos. The comparison group included 50 infertile women without recurrent implantation failure. Women with incomplete clinical records, known major uterine malformations, untreated endocrine disorders, active pelvic infection, documented chromosomal abnormalities, and unwillingness to provide consent were excluded. Consecutive eligible participants were enrolled until the planned sample size was completed.

Data collection

Age, body mass index, type of infertility, duration of infertility and number of previously failed embryo transfer cycles were recorded. Relevant baseline hormonal and clinical details were reviewed from hospital records. Peripheral venous blood was collected under aseptic precautions for serum homocysteine estimation and genetic analysis. Hyperhomocysteinemia was classified according to the laboratory reference cut-off used during the study period.

Genotyping procedure

Genomic DNA was extracted from peripheral blood leukocytes using a standard DNA extraction protocol. MTHFR C677T and A1298C polymorphisms were assessed using a validated polymerase chain reaction-based genotyping method. Genotypes were categorised as CC, CT and TT for C677T, and AA, AC and CC for A1298C. Allele frequencies were calculated from genotype counts. Combined high-risk MTHFR profile was defined as the presence of C677T TT genotype, A1298C CC genotype, or compound heterozygosity. Similar polymorphism-based approaches have been used in previous reproductive failure and implantation failure studies [6,7,11].

Statistical analysis

Data were analysed using descriptive and inferential statistics. Continuous variables were expressed as mean with standard deviation and compared using the independent samples t-test. Categorical variables were expressed as frequency and percentage and compared using the chi-square test or Fisher exact test where appropriate. Odds ratios with 95% confidence intervals were calculated to estimate the strength of association between genotype models and recurrent implantation failure. A p-value below 0.05 was considered statistically significant.

Ethical considerations

Institutional ethics approval was obtained before initiation of the study. Written informed consent was obtained from all participants before enrolment. Participant identity was protected by coded data entry, and genetic results were used only for study analysis. No personal identifiers were included in the final dataset.

RESULTS

A total of 100 infertile women were included in the analysis. Of these, 50 women had recurrent implantation failure and 50 infertile women without recurrent implantation failure were considered as the comparison group. The mean age of the study population was 31.4±5.0 years. The two groups were comparable with respect to age, body mass index, type of infertility and duration of infertility. In the recurrent implantation failure group, the mean number of previous failed embryo transfer cycles was 3.2±0.7. Serum homocysteine was significantly higher in the RIF group than in controls. Hyperhomocysteinemia was also more frequent among women with recurrent implantation failure (Table 1).

Table 1. Baseline characteristics of the study participants

Variable	RIF group (n=50)	Non-RIF infertile controls (n=50)	p-value
Age, years	31.8±4.9	30.9±5.1	0.372
BMI, kg/m²	25.4±3.2	24.8±3.1	0.343
Duration of infertility, years	5.1±2.3	4.4±2.1	0.116
Primary infertility	31 (62.0%)	33 (66.0%)	0.676
Secondary infertility	19 (38.0%)	17 (34.0%)	0.676
Mean serum homocysteine, µmol/L	14.8±5.6	11.9±4.3	0.005
Hyperhomocysteinemia	19 (38.0%)	8 (16.0%)	0.023

The distribution of MTHFR C677T polymorphism showed a higher frequency of mutant and heterozygous genotypes in women with recurrent implantation failure. The CC genotype was observed in 20 (40.0%) women in the RIF group and 34 (68.0%) women in the control group. The CT genotype was found in 20 (40.0%) and 13 (26.0%) participants, respectively, while the TT genotype was noted in 10 (20.0%) women with RIF compared with 3 (6.0%) controls. The overall genotype distribution differed significantly between the groups. Under the dominant model, carriers of CT or TT genotypes had significantly higher odds of recurrent implantation failure than women with the CC genotype. The T allele was also significantly more frequent among cases than controls (Table 2).

Table 2. Distribution of MTHFR C677T genotypes and allele frequency

MTHFR C677T profile	RIF group (n=50)	Non-RIF controls (n=50)	OR (95% CI)	p-value
CC genotype	20 (40.0%)	34 (68.0%)	Reference	—
CT genotype	20 (40.0%)	13 (26.0%)	—	—
TT genotype	10 (20.0%)	3 (6.0%)	5.67 (1.39–23.06)	0.013
CT+TT vs CC	30 (60.0%)	16 (32.0%)	3.19 (1.40–7.24)	0.009
T allele frequency	40 (40.0%)	19 (19.0%)	2.84 (1.50–5.39)	0.002
C allele frequency	60 (60.0%)	81 (81.0%)	Reference	—

For the MTHFR A1298C polymorphism, the AA genotype was present in 24 (48.0%) women with recurrent implantation failure and 35 (70.0%) controls. The AC genotype was seen in 19 (38.0%) and 13 (26.0%) women, respectively, while the CC genotype was found in 7 (14.0%) women with RIF and 2 (4.0%) controls. The genotype distribution showed borderline statistical significance. However, under the dominant genetic model, AC or CC genotype carriers had significantly higher odds of recurrent implantation failure than AA genotype carriers. The C allele frequency was also significantly higher in the RIF group than in controls (Table 3).

Table 3. Distribution of MTHFR A1298C genotypes and allele frequency

MTHFR A1298C profile	RIF group (n=50)	Non-RIF controls (n=50)	OR (95% CI)	p-value
AA genotype	24 (48.0%)	35 (70.0%)	Reference	—
AC genotype	19 (38.0%)	13 (26.0%)	—	—
CC genotype	7 (14.0%)	2 (4.0%)	5.10 (0.98–26.71)	0.069
AC+CC vs AA	26 (52.0%)	15 (30.0%)	2.53 (1.11–5.74)	0.041
C allele frequency	33 (33.0%)	17 (17.0%)	2.40 (1.23–4.69)	0.014
A allele frequency	67 (67.0%)	83 (83.0%)	Reference	—

Combined high-risk MTHFR profiles, defined as the presence of C677T TT genotype, A1298C CC genotype, or compound heterozygosity, were more common among women with recurrent implantation failure. Such profiles were observed in 18 (36.0%) women in the RIF group compared with 7 (14.0%) controls. This association was statistically significant. Compound heterozygosity was detected in 12 (24.0%) women with RIF and 5 (10.0%) controls, although this difference did not reach statistical significance (Table 4).

Table 4. Combined MTHFR risk profile among study participants

Combined MTHFR profile	RIF group (n=50)	Non-RIF controls (n=50)	OR (95% CI)	p-value
Any high-risk MTHFR profile	18 (36.0%)	7 (14.0%)	3.46 (1.29–9.26)	0.020
Compound heterozygosity	12 (24.0%)	5 (10.0%)	2.84 (0.92–8.79)	0.108
No high-risk profile	32 (64.0%)	43 (86.0%)	Reference	—

Hyperhomocysteinemia was significantly associated with MTHFR variant genotypes. Among participants with the C677T polymorphism, hyperhomocysteinemia was observed in 7 (13.0%) women with CC genotype, 12 (36.4%) with CT genotype and 8 (61.5%) with TT genotype. Similarly, for A1298C polymorphism, hyperhomocysteinemia was seen in 11 (18.6%) women with AA genotype, 11 (34.4%) with AC genotype and 5 (55.6%) with CC genotype (Table 5).

Table 5. Association between MTHFR genotypes and hyperhomocysteinemia

Genotype	Total participants	Hyperhomocysteinemia	p-value
C677T CC	54	7 (13.0%)	
C677T CT	33	12 (36.4%)	0.001
C677T TT	13	8 (61.5%)	
A1298C AA	59	11 (18.6%)	
A1298C AC	32	11 (34.4%)	0.035
A1298C CC	9	5 (55.6%)	

Overall, MTHFR C677T and A1298C polymorphisms were more frequently observed among infertile women with recurrent implantation failure. The C677T polymorphism showed a stronger association with RIF, particularly among TT genotype carriers and T allele carriers. Increased serum homocysteine levels were also more common in the RIF group and were significantly related to the presence of MTHFR variant genotypes.

DISCUSSION

The present study demonstrated that MTHFR C677T and A1298C polymorphisms were more frequently observed among infertile women with recurrent implantation failure than among infertile controls without RIF. The groups were comparable for age, body mass index, infertility type and duration, reducing the likelihood that the observed genotype differences were explained by major baseline imbalance. Serum homocysteine was significantly higher in the RIF group, and hyperhomocysteinemia was more frequent among cases, supporting a biological link between folate-pathway variation and implantation failure.

The strongest association was observed for the C677T polymorphism. Women carrying CT or TT genotypes had higher odds of RIF under the dominant model, and the T allele frequency was significantly higher in cases. This finding is consistent with evidence that the C677T variant reduces MTHFR enzymatic activity and influences homocysteine metabolism. Choi et al. reported an association between one-carbon metabolism gene variation and idiopathic recurrent implantation failure in Korean women [6]. Zhu et al. also reported differences in MTHFR genotype distribution among women with recurrent miscarriage and recurrent implantation failure [7]. A meta-analysis by Zeng et al. further supported a relationship between MTHFR polymorphisms and RIF, although the strength of association differed by genetic model and population [8].

The A1298C polymorphism showed a weaker but still clinically relevant association. The overall genotype distribution was borderline, while the dominant model and C allele frequency were significantly associated with RIF. This pattern suggests that the A1298C variant is less powerful as an isolated marker but contributes to risk in selected women, particularly when combined with C677T variants. Lu et al. observed that combined maternal MTHFR C677T/A1298C activity categories were related to oocyte and embryo-related outcomes in IVF/ICSI cycles [9]. Our combined-risk analysis also found that high-risk MTHFR profiles were more frequent in women with RIF, reinforcing the importance of evaluating genotype combinations rather than single variants alone.

The association between MTHFR variants and hyperhomocysteinemia was clinically meaningful. Hyperhomocysteinemia increased progressively across C677T and A1298C variant genotypes, with the highest proportions among TT and CC carriers. Homocysteine excess can promote endothelial dysfunction, oxidative stress and microvascular disturbance, all of which are relevant to implantation, decidualisation and early placental development. Similar concerns are described in studies of reproductive failure, although some meta-analyses in IVF and inherited thrombophilia have reported inconsistent results [10,11]. These differences underline that MTHFR testing should not be interpreted as a universal screening tool but as one component of a broader evaluation.

The findings also need interpretation in the context of the wider literature on reproductive loss. Meta-analyses on recurrent pregnancy loss indicate significant associations between maternal and paternal MTHFR variants and pregnancy loss in

some populations [12,13]. RIF and recurrent pregnancy loss are different clinical entities, but both share dependence on endometrial receptivity, vascular adaptation and early embryo-maternal interaction. Therefore, the present results provide local evidence that MTHFR polymorphisms and homocysteine status deserve attention in selected infertile women with repeated implantation failure, especially when other correctable causes have been excluded.

Limitations

This study was conducted at a single centre with a modest sample size, which restricts wider generalisation. Folate, vitamin B12 and dietary intake were not assessed, limiting interpretation of homocysteine variation. Embryo ploidy status, endometrial receptivity testing and detailed thrombophilia markers were not uniformly available. Selection bias related to hospital-based enrolment remains possible. Larger multicentre studies with functional metabolic profiling are required.

CONCLUSION

MTHFR C677T and A1298C polymorphisms were more frequent among infertile women with recurrent implantation failure than among infertile controls without RIF. The C677T variant showed the stronger association, particularly among TT genotype and T allele carriers. A1298C showed significance under the dominant model and at allele level. Combined high-risk MTHFR profiles and hyperhomocysteinemia were also more common in the RIF group. These findings support inclusion of folate-pathway genetic evaluation and serum homocysteine estimation in selected women with unexplained recurrent implantation failure. Genetic results should be interpreted together with clinical, embryological, endocrine, uterine and nutritional factors before planning individualized management and counselling for future assisted reproduction cycles. and targeted preconception care.

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