

Review Article

Detrimental Effects of Methylenetetrahydrofolate Reductase (MTHFR) Gene Polymorphism on Human Reproductive Health: A Review

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Abstract

Methylenetetrahydrofolate Reductase (MTHFR) is an important enzyme of the folate cycle, which is required to convert 5,10-methyltetrahydrofolate into 5-methyltetrahydrofolate (5-methylTHF). 5-methyl THF is a methyl group donor for several cellular methylation processes. It also donates methyl group for the conversion of homocysteine into methionine, the higher concentration of which is toxic. MTHFR gene C677T polymorphism is clinically important polymorphism and the variant MTHFR (A222V) enzyme has reduced activity, hence increasing the requirement for folic acid. Less conversion of folate to 5-methyl-THF due to C677T polymorphism results in a higher plasma concentration of homocysteine (hyperhomocysteinemia). Individuals having C677T polymorphism are susceptible to various diseases, including reproductive problems like male infertility, polycystic ovary syndrome, Recurrent Pregnancy Loss (RPL), Preeclampsia (PE), placental abruption, and adverse pregnancy outcomes. MTHFR C677T polymorphism mimics folate deficiency, and folate is required for DNA synthesis, repair, methylation, and proper chromosome segregation, and all these processes are important for foetal growth and normal development. Methylation and demethylation processes control the gene expression of about 45% of human genes. Impaired methylation influences the expression of genes involved in the regulation of hormones, spermatogenesis, and oogenesis. In males, oxidative stress damages sperm DNA decreases sperm motility, and may impair fertilization capability. In pregnant women, hyperhomocysteinemia increases oxidative stress and inflammation within the placenta, which causes damage to placental tissue, impairs its function, and disrupts foetal development. Further, hyperhomocysteinemia (HHcy) is embryotoxic and neurotoxic and is responsible for congenital anomalies in the foetus. This review supports the idea that MTHFR C677T polymorphism is associated with an increased risk for male infertility, PCOS, RPL, PE, and congenital anomalies. This review may provide a clue toward a better understanding of the correlation between the MTHFR C677T polymorphism and its detrimental effects on human reproductive health.

Introduction

The folate cycle is essential for cellular functions such as DNA synthesis, DNA repair, cellular methylation, and amino acid metabolism. It involves the conversion of folate into various forms, including tetrahydrofolate (THF) and its derivatives, which are required for key biochemical reactions. The methylenetetrahydrofolate reductase (MTHFR) enzyme plays a critical role in folate (vitamin B9) processing. The enzyme MTHFR plays a key role in converting 5,10-methylene-THF (a form of folate) into 5-methyl-THF and regulates the levels of homocysteine, a potentially harmful sulphur-containing amino acid. 5-methyl-THF is used to donate a methyl group for the conversion of homocysteine into methionine in the methionine cycle. Folate and methionine cycles are two metabolic pathways that work together and are linked by the metabolite 5-methyl-THF. S-adenosyl methionine (SAM) is

produced from methionine and is the primary methyl donor in many methylation reactions, including the methylation of DNA, protein, and lipid (Figure 1). This is a vital process for regulating gene expression, normal chromosome segregation, and maintaining normal cell function. MTHFR enzyme is a homotetramer; each subunit is roughly 77 kDa in size, and the weight of the tetrameric enzyme is about 308 kDa. The active site of MTHFR contains the binding site for 5,10-methylene-THF and NADPH, which is required for the reduction reaction. The enzyme also requires vitamin B12 (riboflavin) for its proper function [1].

The MTHFR gene encodes the enzyme MTHFR. Gene is located on chromosome 1, at position p36.3 region, consists of 11 exons, and spans about 24 kb of DNA. Several polymorphisms are reported in the MTHFR gene, but the C677T polymorphism (rs 1801133) is one of the most studied genetic variants due

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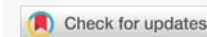
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Keywords: MTHFR; Folate; Homocysteine; Male infertility; Preeclampsia; PCOS; Congenital anomalies



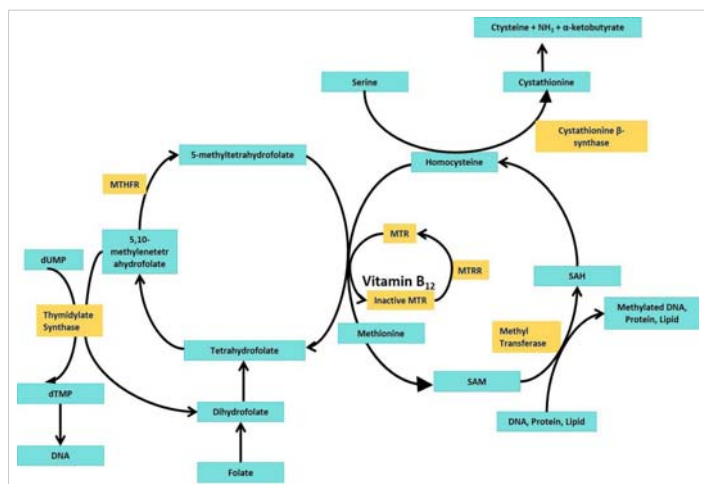


Figure 1: Folate-methionine cycle: MTHFR- methylenetetrahydrofolate; MTR- Methionine synthase; MTRR- Methionine synthase reductase; SAM- S-adenosylmethionine; SAH- S-adenosylhomocysteine; dUMP- deoxyuridinemonophosphate; dTMP- deoxyuridinemonophosphate.

to its impact on folate metabolism. In C677T polymorphism, cytosine (C) is replaced by thymine (T) at position 677 in exon 4, and this substitution causes a change in the amino acid sequence of the MTHFR enzyme (Ala222Val) (Figure 2). The variant MTHFR enzyme (222Val) is thermolabile and exhibits reduced activity compared with the wild type, making it less efficient. C677T polymorphism reduces the enzymatic activity of the MTHFR enzyme, and the variant MTHFR enzyme can impair the conversion of homocysteine to methionine, leading to elevated homocysteine levels in the blood [2,3].

The global frequency of this polymorphism varies significantly by population [4-9]. (The frequency of the T allele in North America, South America, Europe, Africa, Oceania, and Asia was reported as 31.2%, 27.8%, 34.1%, 10.3%, 20.5%, and 19.7% respectively (Figure 3) [9]. The highest frequency of the T allele is reported in Mexico and China (> 35%) (Figure 4).

The detrimental effects of the mutation on the enzymatic activity of MTHFR depend on the status of the folate. The mutation might be tolerated in subjects with a rich folate supply, whereas in individuals with folate insufficiency/ deficiency, it might result in some biochemical or clinical phenotypic consequences [3,10]. Reduced MTHFR activity results in an increased requirement for folic acid to maintain normal homocysteine remethylation.

Individuals with the MTHFR gene C677T polymorphism may be at an increased risk of congenital defects [11-13], metabolic disorders [14], psychiatric disorders [15,16], neurological disorders [17,18], and cancer [19-22]. There is several evidence suggesting that the C677T polymorphism may be associated with reproductive health problems such as male infertility, polycystic ovary syndrome, preeclampsia, Recurrent Pregnancy Loss/ miscarriage (RPL), placental abruption, Preterm Birth (PTB), Intrauterine Foetal Death (IUFD), and congenital anomalies, etc. Hence, in the present

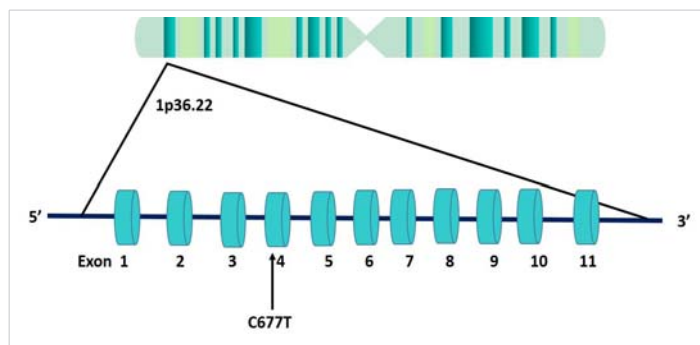


Figure 2: MTHFR gene structure showing the position of C677T polymorphism in exon 4.

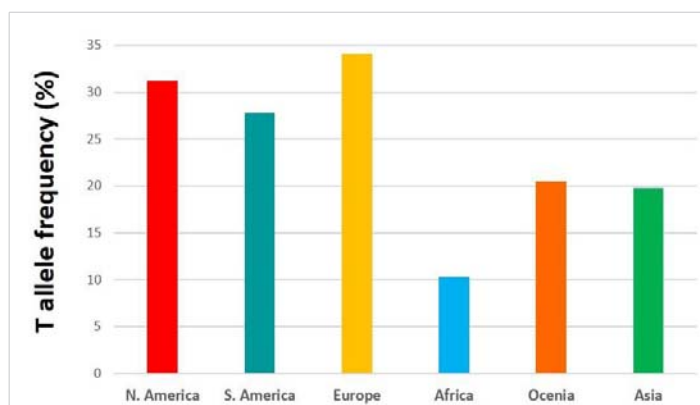


Figure 3: Bar diagram showing T allele frequency in different continents.

review, authors have tried to summarize the detrimental effects caused by the C677T polymorphism on reproductive health.

MTHFR C677T polymorphism and male infertility

Male infertility is a global health concern, affecting 40% - 50% of couples worldwide [23]. It 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, chromosome microdeletions, and gene mutations. About 50% of cases of male infertility are idiopathic. Some common causes of male infertility include: low sperm count, poor sperm motility, abnormal sperm morphology, hormonal imbalances, varicocele, infections, ejaculation issues, environmental factors, and genetic factors.

Spermatogenesis is a very complex event that is controlled by the expression of several genes. Methylation and demethylation play important roles in the production of germ cells. The correct methylation of DNA ensures appropriate chromatin concentration in the sperm head, enabling sperm maturation and its ability in fertilization and post-fertilization events [24]. Numerous studies reported C677T polymorphism as a risk factor for male infertility [10,25-28]. The MTHFR gene polymorphism affects the folate cycle and one-carbon cycle, leading to Hcy accumulation, abnormal DNA methylation, and decreased antioxidant capacity, ultimately affecting sperm quality and causing male infertility [29].

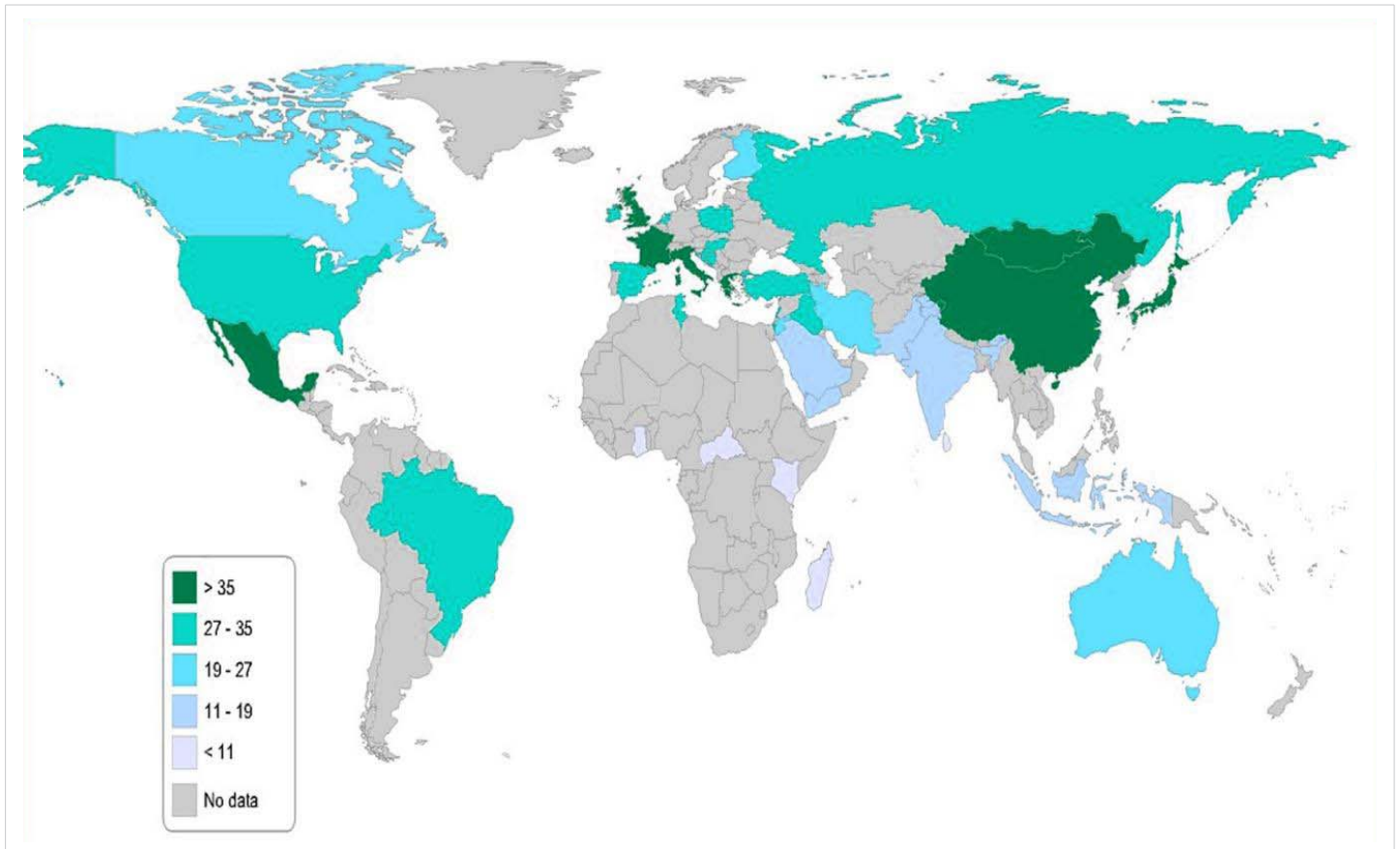


Figure 4: Map showing T allele frequency in different countries of the world.

C677T polymorphism increases homocysteine concentrations in blood (hyperhomocysteinemia (HHcy)), which is reported to be an important risk factor for cardiovascular diseases. HHcy damage to endothelial cells may interfere with penile blood supply or other erection mechanisms [30], leading to ejaculation problems [31,32]. Oxidative stress plays an important role in male infertility [33] and Atig and colleagues [34] reported that about 30% - 80% of male infertility cases are due to Reactive Oxygen Species (ROS)-induced higher oxidative stress. Sperms are particularly intolerant to oxidative stress because, during spermatogenesis, they lose their DNA repair ability. Several studies confirmed that antioxidant supplementation improves semen parameters [35]. Oxidative stress causes lipid peroxidation (peroxidation of polyunsaturated fatty acids of sperm membrane), DNA fragmentation (oxidative modifications), oxidative modifications of proteins, and mitochondrial damage in sperms [36-39]. Oxidative stress can lead to DNA strand breaks and cause genetic mutations, lead to developmental defects in embryos, and a heightened miscarriage risk [38].

Individuals with the T allele have reduced MTHFR activity, so the reduced availability of methylfolate could impair spermatogenesis, leading to sperm DNA fragmentation and poor sperm quality. The C677T polymorphism could influence male fertility in several ways: (i) C677T is associated with

higher levels of homocysteine, which can lead to oxidative stress, oxidative stress damages sperm DNA, decreases sperm motility, and may impair fertilization capability [40], (ii) folate-dependent methylation processes are disrupted, which are critical for spermatogenesis. Supplementation with vitamins B6, B12, and folic acid might improve homocysteine metabolism and reduce oxidative damage, potentially improving sperm function, motility, and viability. Supplementation with antioxidants (e.g., vitamins C, E, and zinc) may help reduce oxidative stress and improve sperm motility and viability.

MTHFR C677T polymorphism and polycystic ovary syndrome (PCOS)

PCOS, also known as polycystic ovary disease (PCOD), is a heterogeneous condition associated with endocrinopathy. PCOS is associated with an increased risk of pregnancy loss with a prevalence of approximately 2% - 25% of women of reproductive age (18 years - 44 years). Stress, obesity, hormonal fluctuations, lifestyle, and genetics are the major risk factors for PCOS. About 70% of PCOS females are infertile [41]. Besides ovarian cysts, several other conditions are also associated with PCOS, like dyslipidaemia, hypertension, type 2 Diabetes, hepatic steatosis, hyperandrogenism, hirsutism, depression, anxiety, etc. It may also cause endometrial carcinoma. PCOS is a polygenic and multifactorial disorder. Cooper and colleagues [42] first reported the genetic basis



of PCOS. Since then, candidate gene variants are reported as risk factors for PCOS susceptibility, such as – CYP11a, CYP17, CYP19, CYP21, PCOS1, FTO, AR, LH, FSHR, INS, IRS1, IRS2, NCOR1, PPARG1, and MTHFR, etc.

Hyperhomocysteinemia (HHcy) in PCOS patients is frequently reported, and MTHFR polymorphism is an important genetic cause for elevated homocysteine concentration, several experimental and clinical pieces of evidence suggested that disturbances in the folate-methionine cycle resulted in ovarian dysfunctions. First of all, Glueck and colleagues [43] have reported an association between the MTHFR C677T polymorphism and PCOS. After that, numerous studies reported C677T polymorphism as a risk factor for PCOS [44,45]. In a recent meta-analysis, authors reported a significant association between the T allele and PCOS risk (OR = 1.31; 95% CI 1.07 -1.62; $p = 0.008$) [46]. The C677T polymorphism leads to reduced activity of the MTHFR enzyme, which results in an impaired ability to process folate and homocysteine.

In summary, MTHFR C677T Might contribute to PCOS by several mechanisms, like-

- i. Folate is essential for DNA synthesis, repair, and methylation and a hypoactive MTHFR affects cell growth and division, which is crucial for reproductive tissues, including the ovaries.
- ii. Impaired methylation influences the expression of genes involved in the regulation of hormones and ovarian function, potentially exacerbating conditions, like PCOS.
- iii. Elevated homocysteine concentration contributes to insulin resistance, a hallmark of PCOS, and endothelial dysfunction, which could worsen metabolic issues related to PCOS.
- iv. Hyperhomocysteinemia causes vascular dysfunction, increased androgen production, and reproductive issues.
- v. Elevated homocysteine levels also contribute to inflammation and oxidative damage in various tissues, including the ovaries, and so worsen the metabolic and reproductive disturbances in PCOS.

MTHFR C677T polymorphism and recurrent pregnancy loss (RPL)/spontaneous abortion (SA)/miscarriage

Recurrent Pregnancy Loss (RPL) is defined as having two or more miscarriages. Risk factors for RPL include uterine problems, hormonal disorders, autoimmune diseases (such as antiphospholipid antibody syndrome), lifestyle and environmental factors, and genetic abnormalities. Most miscarriages that are unexplained, that is idiopathic, are thought to be inherited. The most common genetic factors

contributing to miscarriage are aneuploidy, chromosomal abnormalities in the embryo (translocations), and the presence of candidate gene variants in the mother.

Candidate genes associated with RPL include KIF14, CEP55, STIL, FOXP3, GLE, RYR1, POMT1, DYNC2H1, ALOX15, MMP9, MMP10, TNC, FKBP4, ATAMTS1, FOXA2, FGA, F13A1, KHDC3L, VEGF, ESR, and MTHFR. These genes are often implicated in issues related to cell division, foetal movement, angiogenesis, immune response, and inflammation, which are critical for successful pregnancy development. Numerous studies suggest that women with the MTHFR gene polymorphism may have an increased risk of recurrent pregnancy loss [47-51]. However, some other studies have failed to establish a clear, direct link between MTHFR mutations and miscarriage risk [52-54]. Wen and colleagues [55] have reported a significant association between the C677T polymorphism and spontaneous abortion (OR = 1.43; 95% CI = 1.25 - 1.64, $p < 0.01$).

Reduced MTHFR enzyme activity leads to higher levels of homocysteine in the blood, i.e. hyperhomocysteinemia. Elevated homocysteine levels can cause problems like impaired placental blood flow, increased clotting risk, and inflammation, all of which can interfere with a healthy pregnancy. Higher homocysteine levels can have several negative effects on pregnancy:

- i. Homocysteine has prothrombotic effects; it can increase the likelihood of blood clot formation, and blood clots in the placental vessels can obstruct the blood supply to the fetus, increasing the risk of miscarriage or stillbirth.
- ii. Elevated homocysteine levels can increase oxidative stress and inflammation within the placenta, which causes damage to placental tissue, impairs its function, and disrupts foetal development.
- iii. Homocysteine damages blood vessels and affects the endothelial lining of the blood vessels, which impairs placental blood flow, which is critical for supplying oxygen and nutrients to the developing fetus.
- iv. High homocysteine levels impair ovum quality and lead to difficulties with conception and early pregnancy loss.

The MTHFR mutation may impair the ability to metabolize folate, but supplementation with folate or its active form (5-methyltetrahydrofolate), vitamins B6 and B12, can help lower homocysteine levels and mitigate the negative effects of hyperhomocysteinemia.

MTHFR C677T polymorphism and preeclampsia

The MTHFR C677T polymorphism has been studied for its potential role in preeclampsia (PE) development due to its impact on homocysteine metabolism and folate processing. PE is a pregnancy-related condition characterised by high blood pressure (systolic blood pressure >140 mmHg and diastolic blood pressure >90 mmHg) and proteinuria (>300 mg/day).



The prevalence of PE is approximately 2% - 5%, causing the death of 40,000 pregnant women per annum globally [56]. PE complications start during the second and third trimesters of pregnancy and are life-threatening for both the mother and baby if not properly managed. If PE remains untreated, there is a risk of organ damage, such as the kidneys, often after the 20th week of pregnancy. PE involves the release of angiogenic factors, oxidative stress, hyperlipidaemia, and increased insulin resistance, resulting in sustained inflammation and endothelial dysfunction [57,58]. Several studies have reported MTHFR C677T polymorphism as a potential risk factor for PE [59,60]. Reduced activity of variant MTHFR enzyme reduces the conversion of homocysteine to methionine; hence the homocysteine level is increased. Elevated homocysteine levels are associated with an increased risk of cardiovascular diseases and thrombotic events, which can contribute to the development of preeclampsia by affecting the blood vessels [61]. Elevated homocysteine levels are known to promote oxidative stress, inflammation, and damage to the endothelium (the inner lining of blood vessels). This vascular dysfunction can increase the risk of developing hypertension and preeclampsia. Homocysteine may also interfere with the production of nitric oxide, which is vital for blood vessel dilation and regulating blood pressure.

Placental dysfunction due to folate deficiency and hyperhomocysteinemia is a key feature in the development of preeclampsia. Impaired trophoblastic invasion and poor placental remodelling can lead to reduced blood flow and oxygen delivery to the placenta, resulting in preeclampsia. In the case of preeclampsia, the placenta is unable to supply adequate nutrients and oxygen to the foetus.

Individuals with a single copy of the T allele (heterozygous condition) have mild MTHFR enzyme activity reduction. So the risk of preeclampsia may be slightly higher compared to those with the normal CC genotype. However, women with the TT genotype (mutant homozygous) have more pronounced MTHFR dysfunction, which could potentially increase their risk for preeclampsia, especially if combined with environmental factors such as low folate intake or pre-existing vascular conditions.

The MTHFR C677T polymorphism may cause preeclampsia through its effect on homocysteine levels, which can lead to (i) vascular dysfunction, (ii) reduced production of nitric oxide, (iii) oxidative stress, and (iv) placental insufficiency.

MTHFR C677T polymorphism and adverse pregnancy outcomes

Adverse pregnancy outcomes, including Preterm Birth (PTB), Low Birth Weight (LBW), small-for-gestational-age (SGA), and congenital anomalies, are major determinants for infant morbidity and mortality. PTB and its complications are the leading causes of death among children under 5 years old [62]. Environmental and genetic factors play an important role in the occurrence of PTB, LBW, and SGA [63-66].

Candidate gene studies for PTB and LBW are very few, and the results are inconsistent [67-69]. Two studies, one from India and the other from Japan suggested that the folic acid metabolic enzyme MTHFR C677T polymorphism is a risk for PTB, LBW, and SGA [70]. Kramer, et al. [71] showed that the lower level of folate and hyperhomocysteinemia contributes to adverse pregnancy outcomes.

The MTHFR C677T polymorphism has been associated with an increased risk of preterm birth [72,73]. This may be related to the effects of elevated homocysteine on placental development and function. Hyperhomocysteinemia causes placental abruption, a condition where the placenta detaches prematurely from the uterine wall leading to early delivery in some cases. This can lead to severe bleeding and complications for both the mother and the baby and results in preterm birth or miscarriage. Elevated homocysteine levels damage blood vessels, increase blood clotting, oxidative stress, and inflammation may trigger premature labour [72,73].

The maternal and foetal MTHFR C677T polymorphism has been reported as a risk factor for congenital anomalies such as Neural Tube Defects (NTD) [13], cleft lip and palate [12], and Down syndrome [11], etc. Higher concentrations of homocysteine are teratogenic in the first and mid-trimester [74]. Several evidence suggest that elevated homocysteine levels may interfere with craniofacial development, heart and kidney, etc. Other factors, such as maternal folate status, environmental influences, and additional genetic variations, likely also play a role in the development of congenital anomalies. Mothers with the TT genotype variant (mutant homozygous) are generally at higher risk of DNA hypomethylation, abnormal gene expression, and elevated homocysteine levels and have a higher risk of having a foetus with congenital anomalies. It has been established that the higher concentrations of homocysteine have embryotoxic and neurotoxic effects. It impairs DNA repair in hippocampal neurones and promotes apoptosis [75]. MTHFR polymorphism/folate deficiency and/or hyperhomocysteinemia act prenatally and affect the central nervous system of the foetus through different mechanisms such as (i) folate is required for nucleotide synthesis; its deficiency impairs neural progenitor division and neuron migration, (ii) folate deficiency affects neurogenesis, and (iii) impaired methylation results in abnormal gene expression. Women with the C677T polymorphism should be encouraged to take higher doses of folate, especially in the periconceptual period and early pregnancy. This can help reduce the risk of congenital anomalies and possibly reduce the risk of miscarriage and other adverse pregnancy outcomes.

Conclusion

The MTHFR C677T polymorphism is associated with several detrimental effects on reproductive health, including male infertility, PCOS, miscarriage, preeclampsia, preterm birth, placental abruption, and congenital anomalies. These



risks are often related to impaired folate metabolism and elevated homocysteine levels. Management through folate and antioxidants (Vitamins C, E, and Zinc) supplementation, monitoring of homocysteine levels, and genetic counselling can help mitigate these risks and improve reproductive health outcomes. The takeaway message is that every woman and man of fertile age should get their MTHFR gene checked before pregnancy planning for children for the C677T polymorphism, and if the T allele is present, then they should increase the intake of folate, especially women to avoid recurrent pregnancy loss, preeclampsia, low weight birth, preterm birth, and congenital anomalies, etc.

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