The Role of *MTHFR* Genes in Folic Acid Metabolism (Literature Review)

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ABSTRACT

Background:
Folic acid, a synthetic form of vitamin B_9, is essential for DNA synthesis, red blood cell formation, and the prevention of neural tube defects (NTDs). However, its effectiveness depends on conversion to the biologically active form, 5-methyltetrahydrofolate (5-MTHF), which requires the enzyme methylenetetrahydrofolate reductase (*MTHFR*). Variants in the *MTHFR* gene impair this process and may compromise the utility of folic acid supplementation in affected individuals (Lucock, 2000; Refsum et al., 1998).

Methods:
A comprehensive literature review was conducted, analyzing clinical trials, meta-analyses, molecular studies, and population genetics research. The objective was to evaluate the impact of *MTHFR* gene polymorphisms on folic acid metabolism, homocysteine regulation, and clinical outcomes. Comparative studies on folic acid and methylfolate supplementation were also included.

Results:
Periconceptional folic acid supplementation reduces NTD risk by up to 70%. A pivotal randomized trial reported a 72% reduction in first occurrence of NTDs with folic acid use (Czeizel and Dudas, 1992). However, common *MTHFR* variants such as C677T can reduce enzyme activity by as much as 65%, impairing the conversion of folic acid to 5-MTHF (Frosst et al., 1995; Tsang et al., 2015). Approximately 5–15% of certain populations are homozygous for these mutations, and about 5% of the global population lacks fully functional *MTHFR* activity (Lucock, 2000; van der Put et al., 1998). This leads to elevated homocysteine levels and increased risk for cardiovascular disease, cognitive decline, and adverse pregnancy outcomes (Refsum et al., 1998; Bailey and Gregory, 1999). Methylfolate supplementation bypasses the *MTHFR*-dependent step and is better utilized in individuals with such genetic impairments (Tsang et al. 2015).

Discussion:
Genetic variability in *MTHFR* significantly influences folic acid metabolism (Tsang et al., 2015; Zappacosta et al., 2014). Public health strategies such as folic acid fortification have reduced NTDs (Czeizel and Dudas, 1992; Bailey and Gregory, 1999), but genetic polymorphisms necessitate personalized approaches (Ueland et al., 2001; Lucock, 2000). Supplementation with 5-MTHF may provide improved outcomes in genetically susceptible individuals, underscoring the need for tailored nutritional interventions and consideration of nutrigenetics in public health policies (Prinz-Langenohl et al., 2009; Tsang et al., 2015).

Subjects Genetics, Nutrition **Keywords:** Genetics, Polymorphism, Nutrition, Foli Acid

INTRODUCTION

Folic acid plays a vital role in nucleotide biosynthesis, DNA repair, and methylation reactions. It is particularly crucial during periods of rapid cell division, such as embryogenesis. Public health initiatives promoting folic acid supplementation have significantly reduced the prevalence of neural tube defects worldwide. A landmark randomized controlled trial demonstrated a 72% reduction in first occurrence of NTDs with periconceptional folic acid supplementation (Czeizel and Dudas, 1992). This led to mandatory folic acid fortification in several countries and a 32% reduction in spina bifida incidence in the U.S. (Bailey and Gregory, 1999).

Despite these successes, individual variability in folic acid metabolism exists. Genetic differences, particularly in the *MTHFR* gene, may influence how effectively an individual can convert folic acid into its active form (Lucock, 2000; van der Put et al., 1998; Refsum et al., 1998). Functional polymorphisms in *MTHFR* such as C677T have been associated with reduced enzymatic activity and altered folate status, especially under conditions of low folate intake (Frosst et al., 1995; Tsang et al., 2015). This review explores the biochemical mechanisms of folic acid bioactivation, the role of *MTHFR* polymorphisms, and their impact on clinical and public health outcomes. Understanding these differences is vital for tailoring prevention strategies and optimizing health outcomes through personalized nutrition (Bailey and Gregory, 1999; Lucock, 2000; Refsum et al., 1998; Ueland et al., 2001).

Biochemistry of Folic Acid Metabolism

Folic acid must undergo enzymatic reduction and methylation to become the biologically active form, 5-MTHF. *MTHFR* catalyzes the critical step of converting 5,10-methylenetetrahydrofolate to 5-MTHF (Bailey and Gregory, 1999). This form is essential for the remethylation of homocysteine to methionine, which is vital in DNA methylation and synthesis pathways (Frosst et al., 1995).

Genetic variants that impair *MTHFR* function can lead to hyperhomocysteinemia and related complications (Lucock, 2000; Refsum et al., 1998). The reduction in enzyme activity affects not only folate-dependent metabolic pathways but also increases vulnerability to nutrient deficiencies under low folate intake. Moreover, the efficiency of this conversion varies between individuals depending on their genetic background, affecting the effectiveness of folic acid supplementation programs (Bailey and Gregory, 1999; Ueland et al., 2001).

Notably, C677T and A1298C mutations affect *MTHFR* enzymatic activity, disrupting folate metabolism and reducing cellular availability of 5-MTHF (Tsang et al., 2015; van der Put et al., 1998). This has prompted the consideration of using bioactive folate forms in clinical settings, especially for individuals with reduced enzymatic function.

MTHFR Gene Variants and Their Prevalence

The role of *MTHFR* genes has been well established in scientific literature. As early as 2002, a meta-analysis concluded that individuals with defective *MTHFR* genes may experience folate deficiency even when supplementing with folic acid, due to impaired

activation of the nutrient. This finding has since been supported by more than 300 independent studies involving over 300,000 individuals, making *MTHFR* one of the best-characterized gene-nutrient interactions in the field of nutrigenetics (Ulvik et al., 2007).

C677T and A1298C are the most studied *MTHFR* polymorphisms. Homozygosity for C677T reduces MTHFR enzyme activity by 60–70% (Frosst et al., 1995; Tsang et al., 2015). These mutations are common: 10–15% of Caucasians and Asians are homozygous for C677T, and about 5% globally lack functional *MTHFR* enzyme activity (Lucock, 2000; van der Put et al., 1998; Refsum et al., 1998).

These polymorphisms are associated with impaired folic acid conversion and may influence clinical outcomes, particularly when folic acid intake is inadequate (Qin et al., 2020). Recognizing the distribution and prevalence of these variants is important for developing population-level recommendations that account for genetic susceptibility and for identifying subgroups at higher risk for folate-related disorders (Qin et al., 2020).

Clinical Consequences of Impaired *MTHFR* Function

Reduced *MTHFR* activity results in elevated homocysteine levels, associated with vascular disease, neurodegeneration, and adverse pregnancy outcomes (Refsum et al., 1998; Lucock, 2000; Smith and Refsum, 2016). In hypertensive adults, those with TT genotype demonstrated the highest homocysteine and benefitted most from folic acid supplementation in reducing stroke risk (Qin et al., 2020).

Moreover, mild *MTHFR* deficiency may contribute to cognitive impairment and psychiatric conditions (Bottiglieri, 2005). Further highlighting the systemic relevance of adequate folate status, *MTHFR*-related hyperhomocysteinemia has also been implicated in complications during pregnancy, including preeclampsia and miscarriage, making early detection and intervention through appropriate folate supplementation critical in these populations (Refsum et al., 1998; Lucock, 2000).

Methylfolate as a Targeted Supplementation Strategy

Methylfolate bypasses the *MTHFR*-dependent step, making it effective in individuals with low enzyme activity (Tsang et al., 2015). Cell-based studies demonstrate higher intracellular folate concentrations with 5-MTHF compared to folic acid in individuals with *MTHFR* polymorphisms (Fohr et al., 2002). Additionally, human pharmacokinetic studies have shown that a single dose of [6S]-5-MTHF achieves greater plasma folate exposure and faster absorption than folic acid, without resulting in unmetabolized folic acid in the blood — even in individuals with the *MTHFR* TT genotype (Prinz-Langenohl et al., 2009).

Although more sensitive to degradation, 5-MTHF may be superior for targeted supplementation, especially in genetically predisposed individuals (Lucock, 2000; Lamers, 2011). Clinical studies have shown that methylfolate is safe and effective at improving folate biomarkers and lowering homocysteine, particularly in patients who do not respond well to folic acid (Tsang et al., 2015; Ueland et al., 2001). Its use is increasingly considered in nutrigenetic protocols for personalized supplementation (Bailey and Gregory, 1999; Lucock, 2000).

Table 1: Prominent Human Studies on Folic Acid Interaction with *MTHFR* Gene

Study (Author, Year)	Study Design	Population (Size, Characteristics)	SNP(s)/Focus Investigated	Primary Outcome/Key Findings
Frosst et al., 1995	Genetic association study (variant discovery)	Unselected individuals (general population; allele frequency ~38%)	*MTHFR* C677T	Identified *MTHFR* C677T; TT reduces enzyme activity/thermostability and is linked to higher homocysteine, implicating a vascular risk pathway (Frosst, 1995).
van der Put et al., 1998	Case–control study	NTD cases (n=86) vs controls (n=403), Dutch population	*MTHFR* A1298C and C677T	Discovered A1298C; alone shows limited effect on homocysteine but combined with 677T reduces *MTHFR* activity and is enriched in NTD cases (OR≈2), suggesting added folate-related risk (van der Put, 1998).
Botto & Yang, 2000	HuGE review (systematic review)	Multiple case–control studies worldwide (mothers/infants)	*MTHFR* C677T (±A1298C)	C677T TT modestly increases NTD (spina bifida) risk; low folate amplifies risk and adequate folate mitigates it. Evidence for A1298C alone weaker; combined genotypes may add risk (Botto, 2000).
Ashfield-Watt et al., 2002	Randomized crossover trial	126 healthy adults (42 TT / 42 CT / 42 CC), UK	*MTHFR* C677T	Under low folate, TT shows higher homocysteine; folate-rich diet or 400 µg/day folic acid increases folate and normalizes homocysteine across genotypes (Ashfield-Watt, 2002).
Klerk et al., 2002	Meta-analysis (individual patient data)	40 studies (11,162 CHD cases; 12,758 controls)	*MTHFR* C677T	TT associated with a small ↑ CHD risk overall, seen mainly in low-folate regions and not in folate-fortified settings—consistent with folate status modifying genetic risk (Klerk, 2002).
Tsang et al., 2015	Systematic review & meta-analysis	40 studies (women of childbearing age)	*MTHFR* C677T	TT consistently associated with lower folate biomarkers (serum/RBC), implying higher folate requirement and potential contribution to NTD risk where folate intake is suboptimal (Tsang, 2015).
Colson et al., 2017	Meta-analysis of supplementation trials	10 folic acid intervention studies (≤1.67 mg/day)	*MTHFR* C677T	TT has higher baseline homocysteine/lower folate, but folic acid (400–1670 µg/day, ≥4 weeks) largely eliminates genotype differences in homocysteine (Colson, 2017).
Qin et al., 2020	Randomized controlled trial	20,702 hypertensive adults (CSPPT, China)	*MTHFR* C677T (gene–nutrition interaction)	Enalapril + folic acid reduced first stroke risk, with effects modified by baseline folate/B12 and genotype, supporting a gene–nutrient interaction model (Qin, 2020).

Public Health and Nutrigenetic Considerations

Folic acid fortification has proven effective in reducing NTD incidence, particularly in the U.S. and Canada (Czeizel and Dudas, 1992; Bailey and Gregory, 1999). However, some countries have not implemented fortification, relying on individual supplementation strategies. The absence of universal policies may contribute to persistent folate

insufficiency in populations with low natural dietary folate intake (Lucock, 2000; Refsum et al., 1998).

Given that 5% of the population may not efficiently metabolize folic acid due to *MTHFR* variants, nutrigenetic screening could guide personalized interventions (van der Put et al., 1998; Lucock, 2000). Population-wide policies should consider including education about genetic influences on folate metabolism and ensuring that alternative folate forms like 5-MTHF are accessible to those who may benefit most. Integrating nutrigenetic data into public health policies may enhance outcomes and reduce disease burden, especially among high-risk groups (Lucock, 2000; Lamers, 2011; Zappacosta et al., 2014).

CONCLUSION

MTHFR polymorphisms play a crucial role in determining folic acid metabolism and individual responsiveness to supplementation (Frosst et al., 1995; Lucock, 2000; van der Put et al., 1998). Impairments in *MTHFR* function can lead to decreased bioavailability of active folate and increased homocysteine levels, with wide-ranging implications from cardiovascular to neurocognitive health (Refsum et al., 1998; Smith and Refsum, 2016; Bottiglieri, 2005).

While folic acid fortification remains an effective public health measure, it may not adequately address the needs of individuals with impaired folate metabolism. Supplementation with methylfolate represents a viable, targeted alternative (Tsang et al., 2015; Lamers, 2011). Future strategies should incorporate genetic screening and personalized nutrition to enhance folate-related disease prevention (Bailey and Gregory, 1999; Lucock, 2000). This approach may offer improved clinical outcomes and reduce the burden of folate-related disorders globally. As nutrigenetics continues to evolve, integrating genetic insights into standard nutritional recommendations will be vital for optimizing health outcomes across diverse populations. Ensuring equitable access to bioactive folate forms and supporting education on genetic influences will be key to the next generation of precision nutrition and public health (Bailey and Gregory, 1999; Lucock, 2000).

REFERENCES

- Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med*. 1992;327(26):1832–1835.
- Bailey LB, Gregory JF. Folate metabolism and requirements. *J Nutr*. 1999;129(4):779–782.
- Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet*. 1995;10(1):111–113.
- Ulvik A, Ueland PM, Fredriksen A, Meyer K, Vollset SE, Hoff G, Schneede J. Functional inference of the methylenetetrahydrofolate reductase 677C>T and 1298A>C polymorphisms from a large-scale epidemiological study. *Hum Genet*. 2007 Mar;121(1):57–64.
- Tsang BL, Devine OJ, Cordero AM, Marchetta CM, Mulinare J, Mersereau P, et al. Assessing the association between the methylenetetrahydrofolate reductase (*MTHFR*) 677C>T polymorphism and blood folate concentrations: a systematic review and meta-analysis of trials and observational studies. *Am J Clin Nutr*. 2015 Jun;101(6):1286–1294.
- Lucock M. Folic acid: nutritional biochemistry, molecular biology, and role in disease processes. *Mol Genet Metab*. 2000;71(1–2):121–138.
- van der Put NMJ, Gabreëls F, Stevens EM, Smeitink JA, Trijbels FJ, Eskes TK, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? *Am J Hum Genet*. 1998;62(5):1044–1051.
- Refsum H, Ueland PM, Nygård O, Vollset SE. Homocysteine and cardiovascular disease. *Annu Rev Med*. 1998;49:31–62.
- Smith AD, Refsum H. Homocysteine, B vitamins, and cognitive impairment. *Annu Rev Nutr*. 2016;36:211–239.
- Qin X, Spence JD, Li J, Zhang Y, Li Y, Sun N, et al. Interaction of serum vitamin B12 and folate with *MTHFR* genotypes on risk of ischemic stroke. *Neurology*. 2020 Mar 17;94(11):e1126–e1136.
- Bottiglieri T. Folate, vitamin B12, and neuropsychiatric disorders. *Nutr Rev*. 2005;63(6 Pt 2):S38–S45.
- Prinz-Langenohl R, Brämswig S, Tobolski O, Smulders YM, Smith DE, Finglas PM, Pietrzik K. [6S]-5-methyltetrahydrofolate increases plasma folate more effectively than folic acid in women with the homozygous or wild-type 677C-->T polymorphism of methylenetetrahydrofolate reductase. *Br J Pharmacol*. 2009 Dec;158(8):2014–21.
- Lamers Y. Indicators and methods for folate, vitamin B-12, and vitamin B-6 status assessment in humans. *Curr Opin Clin Nutr Metab Care*. 2011 Sep;14(5):445–454.
- Lucock M, Yates Z. Folic acid fortification: a double-edged sword. *Curr Opin Clin Nutr Metab Care*. 2009;12(6):555–564.
- Zappacosta B, Graziano M, Persichilli S, Di Castelnuovo A, Mastroiacovo P, Iacoviello L. 5,10-Methylenetetrahydrofolate reductase (*MTHFR*) C677T and A1298C polymorphisms: genotype frequency and association with homocysteine and folate levels in middle-southern Italian adults. *Cell Biochem Funct*. 2014 Jan;32(1):1–4.
- Ueland PM, Hustad S, Schneede J, Refsum H, Vollset SE. Biological and clinical implications of the *MTHFR* C677T polymorphism. *Trends Pharmacol Sci*. 2001;22(4):195–201.
- Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. Am J Epidemiol. 2000;151(9):862–77.
- Ashfield-Watt PA, Pullin CH, Whiting JM, Clark ZE, Moat SJ, Newcombe RG, et al. MTHFR 677C→T genotype modulates homocysteine responses to a folate-rich diet or low-dose folic acid supplement: a randomized controlled trial. Am J Clin Nutr. 2002;76(1):180–6.
- Klerk M, Verhoef P, Clarke R, Blom HJ, Kok FJ, Schouten EG, et al. MTHFR 677C→T polymorphism and risk of coronary heart disease: a meta-analysis. JAMA. 2002;288(16):2023–31.
- Colson NJ, Naug HL, Nikbakht E, Zhang P, McCormack J. The impact of MTHFR 677C/T genotypes on folate status markers: a meta-analysis of folic acid intervention studies. Eur J Nutr. 2017;56(1):247–60.