

Review

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Methylenetetrahydrofolate reductase and transcobalamin genetic polymorphisms in human spontaneous abortion: biological and clinical implications

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Abstract

The pathogenesis of human spontaneous abortion involves a complex interaction of several genetic and environmental factors. The firm association between increased homocysteine concentration and neural tube defects (NTD) has led to the hypothesis that high concentrations of homocysteine might be embryotoxic and lead to decreased fetal viability. There are several genetic polymorphisms that are associated with defects in folate- and vitamin B₁₂-dependent homocysteine metabolism. The methylenetetrahydrofolate reductase (MTHFR) 677C>T and 1298A>C polymorphisms cause elevated homocysteine concentration and are associated with an increased risk of NTD. Additionally, low concentration of vitamin B₁₂ (cobalamin) or transcobalamin that delivers vitamin B₁₂ to the cells of the body leads to hyperhomocysteinemia and is associated with NTD. This effect involves the transcobalamin (TC) 776C>G polymorphism. Importantly, the biochemical consequences of these polymorphisms can be modified by folate and vitamin B₁₂ supplementation. In this review, I focus on recent studies on the role of hyperhomocysteinemia-associated polymorphisms in the pathogenesis of human spontaneous abortion and discuss the possibility that periconceptual supplementation with folate and vitamin B₁₂ might lower the incidence of miscarriage in women planning a pregnancy.

Homocysteine metabolism and genetic polymorphisms – an overview

Accumulating evidence suggest that the sulfur-containing amino acid homocysteine plays a role in various developmental disorders [1]. Two main factors affect homocysteine concentration in humans: diet (mainly intake of folate and vitamin B₁₂) and polymorphisms in genes that encode enzymes or transport proteins involved in folate- and vitamin B₁₂-dependent homocysteine metabolism, the so called one-carbon metabolism, which is a complex series of metabolic pathways crucial for DNA synthesis

and repair and a wide range of methylation reactions (Fig. 1).

Methylenetetrahydrofolate reductase (MTHFR, EC 1.5.1.20) is a key enzyme in one-carbon metabolism. The enzyme catalyzes the conversion of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, the predominating circulating form of folate. 5-methyltetrahydrofolate participates in the vitamin B₁₂-dependent remethylation of homocysteine to methionine that is converted to S-adenosylmethionine that serves as a methyl group donor in the methylation of DNA, proteins,

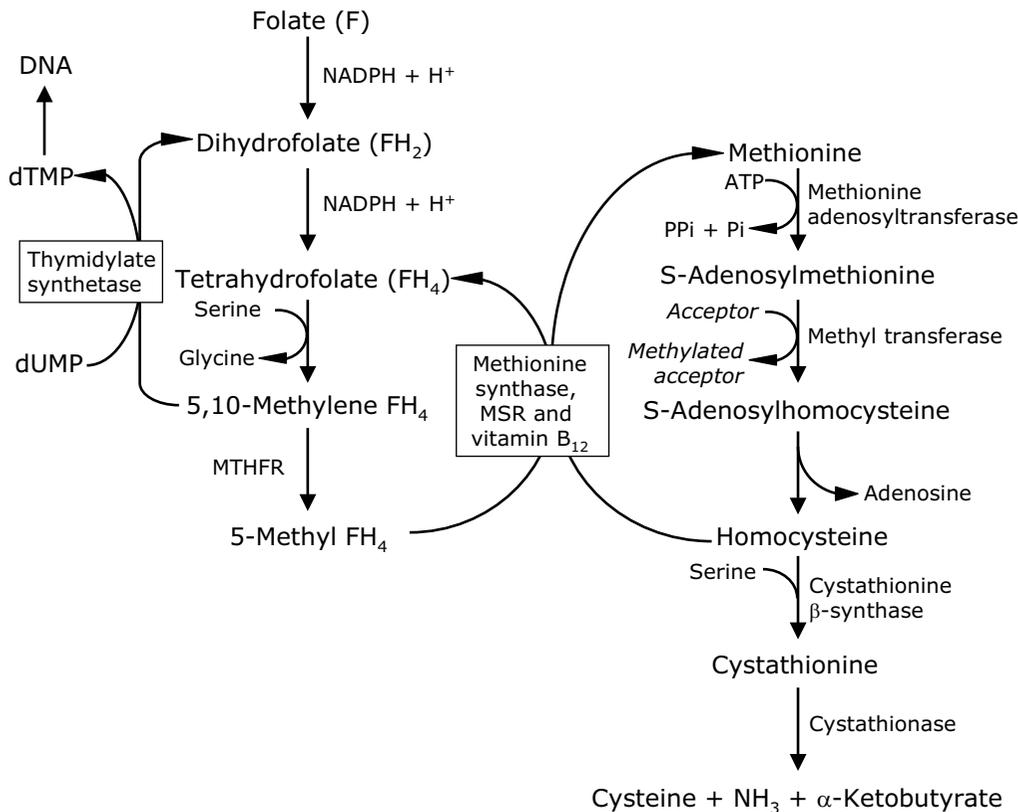


Figure 1

Overview of the human folate- and vitamin B₁₂-dependent homocysteine metabolism indicating its one-carbon donors and acceptors involved in methyl group biogenesis and DNA synthesis. MTHFR, methylenetetrahydrofolate reductase; MSR, methionine synthase reductase; dUMP, deoxyuridylate; dTMP, deoxythymidylate.

neurotransmitters and phospholipids [2]. MTHFR gene polymorphisms are commonly associated with hyperhomocysteinemia [3,4]. In pregnant women this is a risk factor for neural tube defects (NTD) [5,6] and recurrent embryo loss [7-9] as discussed thoroughly in three recent reviews [10-12]. Recently, Gris *et al.* also reported an association between increased levels of homocysteine and a first early pregnancy loss [13]. The best characterized MTHFR genetic polymorphism consists of a 677C>T transition which results in an alanine to valine substitution in the predicted catalytic domain of MTHFR [3]. This substitution renders the enzyme thermolabile, and homozygotes and heterozygotes have about a 70% and 35% reduced MTHFR activity *in vitro*, respectively. Homozygosity for the 677T allele is associated with elevated homocysteine levels, predominantly in individuals who have a

low plasma folate level [14]. Furthermore, the level of plasma homocysteine can be lowered in homozygous individuals by folate supplementation [15]. About half the general population carries at least one mutated allele and the frequency of the homozygous mutated genotype (677TT) ranges from 1 to 20% depending on the population [16]. A second common polymorphism in the MTHFR gene is a 1298A>C transition which results in a glutamate to alanine substitution within a presumed regulatory domain of MTHFR [4,17]. The 1298C allele has been reported to lead to decreased enzyme activity, although not to the same extent as the 677T allele [4,17,18]. Individuals who are compound heterozygous for the 677T and 1298C alleles, which produces a 677CT/1298AC genotype, have according to some studies 40–50% reduced MTHFR activity *in vitro* and a biochemical

profile similar to that seen among 677T homozygotes with increased homocysteine levels and decreased folate levels [4,17]. However, recent results indicate that the MTHFR 1298A>C polymorphism does not contribute significantly to hyperhomocysteinemia, neither by itself nor in combination with the 677C>T polymorphism [19,20], and the phenotypic effect of the polymorphism has also been questioned from a biochemical point of view [21]. It must, however, be borne in mind that the absence of a biochemical phenotype *in vitro* does not necessarily rule out the possible importance of the 1298A>C polymorphism *in vivo*, for instance during times of high folate requirement, such as pregnancy [22].

Yet another common genetic polymorphism that influences homocysteine concentration is a C>G transition at position 776 (776C>G) in the transcobalamin (TC) gene [23]. The transition results in a substitution of arginine for proline at codon 259 and is the major determinant of the TC phenotypic variability in Caucasian populations [24,25]. TC is the critical transporter that delivers vitamin B₁₂ to peripheral tissues and heterozygosity for the polymorphism has been associated with hyperhomocysteinemia [25]. The wild-type 776C allele has positive effects on TC plasma levels [25-27], which may be important for efficient vitamin B₁₂ delivery to the cells of the growing embryo.

Folate, vitamin B₁₂ and homocysteine in embryonic development

One-carbon metabolism during embryonic development has been studied mostly with regard to the development of the nervous system. Pregnant women who are folate deficient have a greatly increased risk of NTD in their babies and periconceptional folate supplementation protects against this effect [12,28-31]. The molecular events that lead to NTD due to folate deficiency are not known but may include insufficient methylation of crucial metabolites in the developing embryo and/or abnormalities in neural cell proliferation, differentiation and apoptosis, which may be due to DNA nucleotide misincorporation that accompanies folate deficiency in proliferating cells [2]. It seems likely that these fundamental events also might lead to decreased fetal viability. In agreement with this view, a recent population-based case-control study showed an increased risk of early spontaneous abortion among pregnant women with low plasma folate levels [32]. Vitamin B₁₂ deficiency during pregnancy results in elevated homocysteine concentration in the embryo and increases the incidence of NTD [26,31,33,34]. In addition, hereditary deficiency of transcobalamin results in profound neurological abnormalities and mental retardation [35]. Three studies envisage a direct embryotoxic effect of homocysteine [36-38]. In one study exposure of chick embryos to homocysteine

resulted in defects both of the heart and the neural tube [36], while exposure of mouse and rat embryos to homocysteine in two other studies mainly resulted in growth retardation and abnormalities of somite development, but not in neurulation defects or other teratogenic effects [37,38]. The precise mechanism of homocysteine toxicity remains elusive but there are several hypotheses, some of which have been tested experimentally. The toxic effect of homocysteine in developing rat embryos may result from increased formation of S-adenosylhomocysteine that could inhibit critical methylation reactions [38]. Elevated homocysteine concentration could also inhibit *de novo* synthesis of deoxythymidylate (dTMP). Exposure of proliferating B-lymphoid Raji cells to excess homocysteine or methionine increases the uptake of exogenous thymidine owing to inhibition of the thymidylate synthase-catalyzed reaction in which deoxyuridylate (dUMP) is converted to dTMP (Fig. 1) [39]. It is thought that 5,10-methylenetetrahydrofolate, a cofactor in this reaction, is depleted in the presence of excess homocysteine due to increased demand for 5-methyltetrahydrofolate to remove homocysteine by methylation. This might induce DNA damage through increased misincorporation of dUMP in place of dTMP in DNA followed by excision-repair reactions, DNA strand breaks, cell-cycle arrest and, ultimately, apoptosis. In conclusion, folate, vitamin B₁₂ and homocysteine play several fundamental roles in growing cells and thus in the developing embryo. It is also possible that homocysteine by itself induces some of the developmental disorders previously attributed to folate and/or vitamin B₁₂ deficiency.

MTHFR and TC genetic polymorphisms in human spontaneous abortion

Through their defects in folate- and vitamin B₁₂-dependent homocysteine metabolism, MTHFR and TC polymorphisms have been implicated as risk factors for several developmental disorders, such as NTD [40-44], orofacial clefting [45] and Down syndrome [46], although there are studies failing to replicate these findings [47-51]. Interestingly, Wenstrom *et al.* found a strong association between fetal MTHFR 677T alleles, elevated homocysteine concentration in amniotic fluid and neurotubular defects spanning the cervical-lumbar spine, lumbosacral spine, and occipital encephalocele, but not anencephaly, exencephaly or spina bifida confined to the sacrum [52]. The authors conclude that the MTHFR 677T allele predisposes only to certain types of NTD [52].

As mentioned earlier, maternal hyperhomocysteinemia is a risk factor for recurrent embryo loss [7-9] and also for a first early embryo loss [13]. In addition to these findings, some studies have reported an association between maternal MTHFR 677T alleles and increased risk of recurrent spontaneous abortion [53-56] (Table 1). However, this effect has not been replicated in other studies [57-63].

Table 1: Maternal and fetal MTHFR 677T alleles in human spontaneous abortion.

REFERENCE	NUMBER OF PARTICIPANTS CASES/CONTROLS	RESULT
<i>Recurrent spontaneous abortion</i>		
<i>Maternal genotypes</i>		
Foka <i>et al.</i> [58]	80/100	No risk
Holmes <i>et al.</i> [59]	173/67	No risk
Kumar <i>et al.</i> [56]	24/24	Increased risk
Kutteh <i>et al.</i> [60]	50/50	No risk
Lissak <i>et al.</i> [55]	41/18	Increased risk
Murphy <i>et al.</i> [61]	40/540	No risk
Nelen <i>et al.</i> [53]	185/113	Increased risk
Pihusch <i>et al.</i> [62]	102/128	No risk
Wramsby <i>et al.</i> [63]	84/69	No risk
<i>Non-recurrent spontaneous abortion</i>		
<i>Maternal genotypes^a</i>		
Alfirevic <i>et al.</i> [64]	18/44	No risk
Gris <i>et al.</i> [65]	232/464	No risk
Kupferminc <i>et al.</i> [66]	12/110	No risk
Many <i>et al.</i> [67]	40/80	No risk
Martinelli <i>et al.</i> [68]	67/232	No risk
Murphy <i>et al.</i> [61]	24/540	No risk
<i>Spontaneous abortion</i>		
<i>Fetal genotypes</i>		
Isotalo <i>et al.</i> [69]	161/119	Increased risk ^b
Zetterberg <i>et al.</i> [22]	80/125	Increased risk ^b
Zetterberg <i>et al.</i> [71]	76/114	Increased risk ^c

^a Five of these six studies examined late fetal loss (fetal death after 19 weeks or more of gestation) ^b Interaction between fetal MTHFR 677T and 1298C alleles ^c Interaction between fetal MTHFR 677T and TC 776G alleles

Likewise, six studies found no association between maternal 677T alleles and non-recurrent fetal loss [61,64-68], but five of these only examined late fetal loss (fetal death after 19 weeks or more of gestation). Aside from strong bias toward late-pregnancy outcomes and nutritional and ethnic differences between the study populations, one explanation for the discrepant results may be that the numbers of study participants have been relatively small, yielding a low power, i.e. a low probability for detecting a difference if there is one. It is also possible that fetal genotypes would have produced clearer results. The common effects of MTHFR and TC mutated alleles are lower bioavailability of folate and vitamin B₁₂ and increased homocysteine concentration. Since vital cellular processes such as proliferation and differentiation are dependent on folate- and vitamin B₁₂-mediated one-carbon metabolism, these effects may be especially pronounced early in embryogenesis when the cells undergo rapid proliferation and differentiation [12]. Hence, it would be of special interest to analyze spontaneously aborted embryos, not just the mothers, for the MTHFR and TC polymorphisms. Recently, four studies addressing this issue were undertaken [22,69-71] (Table 1). Isotalo *et al.* reported high

prevalence of mutated MTHFR alleles in aborted embryos [69]. However, their study group consisted of fetal tissue samples from both spontaneous and therapeutic terminations of pregnancy, which diminishes the interpretability of the investigation. We undertook a similar study, except that only spontaneously aborted embryos (fetal death between sixth and twentieth week after conception) were included. There was a significant odds ratio for spontaneous abortion of 14.2 (95% CI 1.78-113; p = 0.001) when comparing the prevalence of one or more 677T and 1298C alleles versus the wild type combined genotype (677CC/1298AA) in cases and controls, indicating that the MTHFR polymorphisms may have a major impact on fetal survival [22]. The prevalence of the mutated TC 776G allele was significantly increased in aborted embryos while the frequency of wild-type TC 776C homozygotes was much lower among spontaneously aborted embryos than controls (9.1% and 32.2%, respectively; p < 0.001) [70]. These data are consistent with the view that the 776C allele may have beneficial influences during embryogenesis, conceivably through its positive effect on vitamin B₁₂ intracellular bioavailability, which among other things results in reduced homocysteine concentration. Finally,

we addressed the possibility of a gene-gene interaction between the MTHFR and TC polymorphisms in human spontaneous abortion [71]. Embryos that had combined MTHFR 677TT and TC 776CG or 776GG genotypes; genotypes that individually are associated with impaired homocysteine metabolism in adults, were at increased risk for spontaneous abortion compared to embryos that had only one of these genotypes. This indicates a detrimental interaction between the hyperhomocysteinemia-associated MTHFR 677TT and TC 776CG or 776GG genotypes during embryogenesis and further underscores the linkage between decreased fetal viability and elevated homocysteine concentration. Other candidate gene-gene interactions that remain to be explored in spontaneous abortion are between the MTHFR and TC genes and the genes for cystathionine β -synthase [72], methionine synthase [42] and methionine synthase reductase [73], which all are involved in one-carbon metabolism and have been examined in relation to NTD.

Another possible, but as yet unexplored, type of genetic interaction in human spontaneous abortion is a maternal-fetal interaction. The 677C>T polymorphism confers an even higher risk for NTD if both the mother and her child are homozygous for the 677T allele, as compared to if only the mother or the child is homozygous [42]. Conceivably, the homocysteine concentration in the embryo would be even higher if both the mother and the embryo carried hyperhomocysteinemia-associated MTHFR and TC genotypes and, if the hypothesis of the embryotoxicity of homocysteine were true, this would lead to even further increased risk of spontaneous abortion.

Prevention of human spontaneous abortion by periconceptual B-vitamin supplementation

The MTHFR and TC polymorphisms are modifiable genetic risk factors. Increased intake of folate [15] and vitamin B₁₂ (Zetterberg *et al.*, unpublished data) neutralizes the negative effects of the mutated alleles on homocysteine metabolism. The effect of folate supplementation on prevention of NTD has been questioned [74], but generally it appears that daily consumption of 400 μ g of folate before conception and during early pregnancy reduces the occurrence of NTD [29,30]. Moreover, the impact of mandatory fortification of grain with folate on the prevention of NTD was recently documented in the United States where a 19% reduction in NTD birth prevalence was seen following folate food fortification, although factors other than fortification may have contributed to this decline [75]. It has been suggested that the probable reduction in risk of pregnancy complications may be more effective if a combination of folate and vitamin B₁₂ is given [31,76]. Hence, pregnant women that carry hyperhomocysteinemia-associated MTHFR and TC genotypes might benefit from supplementation with both

folate and vitamin B₁₂ to reduce the risk of miscarriage. Since the MTHFR and TC polymorphisms are very common, and since the data indicate that the fetal genotype is as important as the maternal genotype, a general recommendation of periconceptual supplementation with folate and vitamin B₁₂ may be considered. It should, however, be remembered that certain populations already have adequate intake of folate and vitamin B₁₂ even for carriers of variant alleles. For instance traditional Mediterranean diet is abundant in folate [77,78].

Can periconceptual B-vitamin supplementation lead to any adverse effects? There is evidence for an increase in twinning frequency with increased risk of pregnancy complications following folate supplementation [79,80]. Moreover, a case-control study investigated the 677C>T polymorphism in mothers with dichorionic twin pregnancies and found a lower frequency of the 677T allele amongst mothers of twins compared with women who gave birth to singletons [81]. The authors suggest that the MTHFR 677T allele is protective against multiple pregnancies and that folate supplementation might increase the risk of twinning. However, recent results from a large population-based cohort study in China showed no evidence for effects of folate supplementation on twinning frequency [82]. Likewise, there was no evidence for association between MTHFR genotype and twinning in mothers of twins or for the loss of specific MTHFR genotypes during twin pregnancies in a very recent large study of families with twins and of twin pairs [83]. Lastly, there has been some concern that folate supplements might increase abortion rates by delaying very early abortions that would not have been recognized as a pregnancy in the absence of vitamin supplementation [84,85], but a large population-based case-control study revealed no association between high folate levels and increased risk of spontaneous abortion [32]. In fact, a non-significant trend toward a protective effect associated with high folate levels was seen.

Taken together, the data reviewed in this article warrant additional investigations exploring the potential beneficial effects of periconceptual supplementation with both folate and vitamin B₁₂ in the prevention of spontaneous abortion.

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