

METHYLENETETRAHYDROFOLATE REDUCTASE POLYMORPHISMS AS POSSIBLE RISK FACTORS OF VENOUS THROMBOSIS – TOO WEAK TO TAKE CARE, TOO FREQUENT TO BE IGNORED...

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ABSTRACT

Methylenetetrahydrofolate reductase (MTHFR) is an enzyme involved in the metabolism of homocysteine. It has been found that an increased concentration of homocysteine in circulating blood (hyperhomocysteinaemia) may lead to blood vessel damage, which may further be associated with higher risk of various cardiovascular disorders, including venous thrombosis (VT). Among various factors identified to be responsible for hyperhomocysteinaemia, an important role is played by the genetically determined decrease of MTHFR enzymatic activity. This decrease may result from several nucleotide polymorphisms (SNPs) of the gene encoding for MTHFR, with the two most important SNP variants: C677T and A1298C. However, the exact role of the mentioned polymorphisms in the pathogenesis of venous thrombosis remains unclear, especially since the results of several studies conducted so far were inconsistent and did not confirm univocally any such direct relationship. In this review the authors aim to explain the reason of such a discrepancy. Moreover, the authors try to answer the question, whether both mentioned polymorphic variants of the MTHFR gene (C677T and A1298C) are in fact considerable risk factors for venous thrombosis. Based on various pro and contra published so far, the authors conclude that polymorphisms of MTHFR, although recognised as rather weak single risk factors of VT, cannot be underestimated and still require our attention.

Key words: homocysteine, MTHFR, polymorphism, SNP, thrombosis.

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INTRODUCTION

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme involved in homocysteine (HCy) metabolism [1]. It converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The latter compound serves as the donor of methyl groups for the vitamin B₆- and B₁₂-dependent remethylation of homocysteine to methionine. It is noteworthy that the folate pathway is also involved in the generation of purines, which are necessary for the synthesis of RNA and DNA molecules [2]. Homocysteine is metabolised in the kidney by an alternative pathway of transsulphuration, where in the first step HCy is condensed with serine to form cystathionine, which is then broken down into cysteine, followed by its further conversion into taurine and sulphate (Fig. 1).

HYPERHOMOCYSTEINAEMIA

In normal conditions both pathways are usually sufficient to maintain the HCy serum concentration below the level of 12-15 µmol/l. However, kidney insufficiency, deficiency of folic acid, vitamins B₆ and B₁₂, or any dys-

function of enzymes involved in HCy metabolism may result in hyperhomocysteinaemia [3]. According to the American Heart Association, serum concentrations of 15-31 µmol/l were considered as mildly increased, 31-100 µmol/l as intermediately increased, and values > 100 µmol/l were recognised as severe hyperhomocysteinaemia [4].

It has been demonstrated in both, *in vitro* and *in vivo* systems, that high concentration of HCy cause damage of endothelial cells, inhibited expression of endothelial nitric oxide synthase, induce oxidative stress, enhance leukocyte recruitment, activate matrix metalloproteinases (MMP)-9, and promote proliferation of smooth myocytes [5, 6]. Thus, hyperhomocysteinaemia is currently recognised as an important risk factor of several cardiovascular disorders, including atherogenesis and thrombosis. Regarding the latter, it was estimated that the HCy serum concentration above 16.6 µmol/l may be responsible for a 2- to 5-fold higher risk of venous thrombosis (VT) [7-9].

As mentioned previously, hyperhomocysteinaemia may be due to abnormal activity of enzymes engaged in HCy metabolism, mainly MTHFR. Apart from folate and

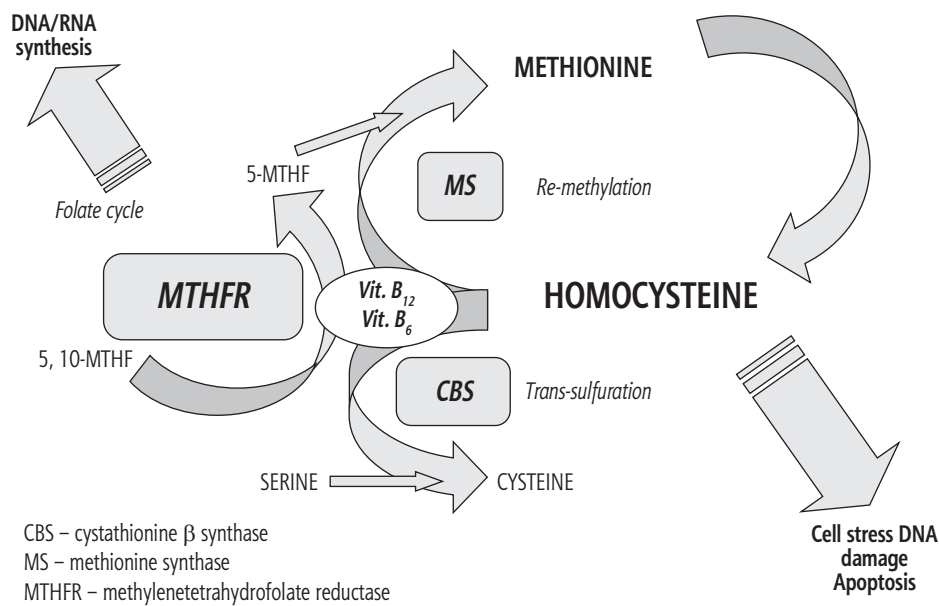


Fig. 1. Schematic representation of the main metabolic pathways involved in homocysteine metabolism (description in text)

vitamin B deficiencies, decreased activity of MTHFR may result from some molecular variations of gene encoding for this enzyme, characterised as single nucleotide polymorphisms (SNPs).

MTHFR POLYMORPHISMS

The human MTHFR gene is composed of 20 kilobase pairs, organised in 11 exons and located in chromosome 1p36.3 [10, 11]. Until now there have been over 40 identified SNPs associated with this gene; however, two of them seem to be the most clinically relevant functional variants: C667T in exon 4 and A1298C in exon 7 [12].

C677T

The most common MTHFR polymorphism (C677T) leads to substitution of an alanine by a valine residue. This change results in thermolability of the enzyme and affects its catalytic activity at 37°C [13], followed by suppression of remethylation pathway and increased risk of hyperhomocysteinaemia. Indeed, it has been proven that individuals bearing the CT genotype had approximately 65% of wild-type enzyme activity, with plasma concentrations of Hcy 3-12% higher than those with the CC genotype. Furthermore, in TT homozygotes MTHFR activity reached 30% of the CC homozygotes, whereas Hcy levels were 11-32% higher as compared to wild-type homozygotes [14-17]. It is noteworthy that both, CT and TT genotypes do not necessarily lead to increased Hcy levels, especially with a sufficient folate supply [18]. Conversely, deficiencies of the folate and vitamin B₆ and B₁₂ in these individuals may result in intermediate or severe hyperhomocysteinaemia [19, 20].

It has been demonstrated that MTHFR C677T SNP was associated with a significant risk of neural tube defects in the developing foetus, as well as repetitive miscarriages and cardiovascular disorders in adults [1, 21-23]. However, despite proven correlation between the above-mentioned risk of cardiovascular disorders and 677T allele prevalence, the contribution of this MTHFR variant to the increased risk of vein thrombosis remains equivocal. The results of studies focused on that issue have shown large discrepancies, which may be due to a highly heterogeneous distribution of MTHFR SNP worldwide [24-26]. The frequency of 677T allele was reported as being low in Africans (6-10%), relatively high in Caucasians (17-50%), and highest in the Mexican population (over the 50%) (Fig. 2) [27]. Interestingly, there is a north-to-south increase in the frequency of this allele in Europe and North America, whereas a reverse trend has been observed in China, India, and Pakistan [28]. Of note, several studies have shown a negative correlation between 677T occurrence and longevity in various populations. Thus, since the presence of 677T variant increases the risk of cardiovascular problems (including those with fatal outcome), due to a kind of negative selection its prevalence in older patients is relatively low, as compared to the general population [29-31].

A1298C

The next clinically relevant MTHFR polymorphism (A1298C) leads to substitution of a glutamate to alanine in the enzyme amino acid sequence. Also, this exchange, although to a lesser extent than for the previously described C677T SNP, was shown to affect enzyme activity and resulted in decreased concentrations of Hcy in the

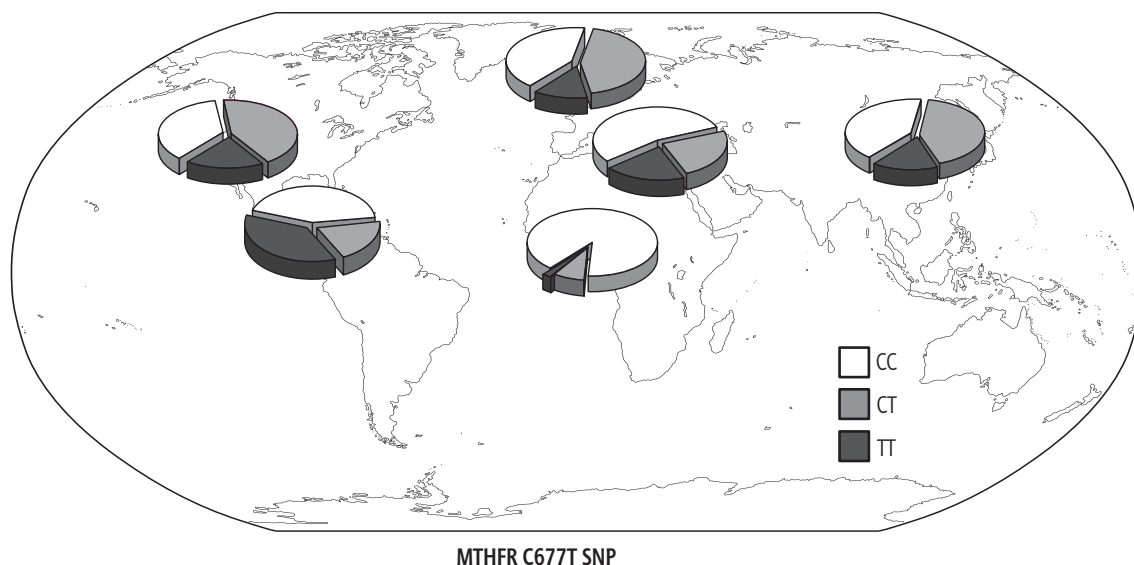


Fig. 2. Worldwide distribution of MTHFR C677T polymorphism (description in text)

serum of patients bearing the 1298C allele [32]. Therefore, this polymorphism was also supposed to be a risk factor for neural-tube defects [33] and congenital heart diseases [34]. Again, similarly to C677T SNP, the association of A1298C SNP with venous thrombosis is controversial.

In contrast to the above-mentioned C677T polymorphism, the distribution of A1298C SNP is poorly recognised, mainly due to its less pronounced effect on enzyme activity. However, the prevalence of 1298C variant in Central Europe seems to be higher than in West Africa, and also, contrary to 677T allele, in Mexico [27, 35]. According to a hypothesis concerning the origin of the observed frequency of MTHFR SNPs among the global population, such a distribution pattern may result from specific gene-nutrient interactions (including different nutritional habits, various exposure to UV radiation, etc.) [27, 36].

METHYLENETETRAHYDROFOLATE REDUCTASE SINGLE NUCLEOTIDE POLYMORPHISMS IN VENOUS THROMBOSIS

Although both of the MTHFR SNPs described above are considered as examples of genetically determined thrombophilia, as mentioned previously, their association with VT remains unclear. This discrepancy is especially obvious, when compared to “classical” representatives – defective variants of coagulation factors – factor V R506Q (better known as factor Leiden), and prothrombin A20210G SNPs, or relatively rare mutations of natural anticoagulants – protein S or protein C. In contrast to them, the relationship between MTHFR SNPs and coagulopathy is indirect and more complex, especially since it is closely related to the dietary and/or metabolic condition of the patient. Furthermore, this analysis is further impeded by the uneven allele distribution among various ethnic groups, which may affect the significance of studies undertaken so far.

Pro

Several studies have suggested an association between MTHFR C677T SNP and increased risk of VT, mainly in Asian populations, including Far Eastern and Middle Eastern countries. Abudurehman *et al.* [37] investigated this connection in VT patients originating from two ethnic groups of Uygur and Han, from the Xinjiang Autonomous Region in China. The frequency of MTHFR 677TT genotype was 28.38% in Uygur VT patients and 27.03% in Han VT patients, versus 12.79% and 14.92% in respective control individuals. Similar results were reported by Yin *et al.* in a case-control study conducted in Shandong Province in China [38]. Also, Jang *et al.* postulated that the presence of MTHFR 677TT genotype was associated with an increased risk of VT in Koreans [39]. The authors also included the MTHFR A1298C in their analysis. However, they did not find any association between the latter SNP and VT occurrence in that population.

Very similar data were obtained by Kreidy *et al.* in Lebanese patients with VT [40]. Again only MTHFR 677T allele, but not 1298C, was associated with the increased risk of morbidity.

Tawfik *et al.* have shown that in the Egyptian population the 677T allele may be considered as a significant risk factor of thrombosis since the TT genotype was present in 51% of VT patients, whereas its frequency in the control group was only 4.1% [41].

The association of MTHFR 677TT genotype with an increased risk of VT was also reported in patients from central Iran [42]. The frequency of TT homozygotes in a VT group was significantly higher (11.1%) than in healthy controls (3.9%). The same observation was published by Kupeli *et al.* in relation to the Turkish population [43]. Moreover, the MTHFR 677T allele was strongly

associated with the occurrence of pulmonary embolism in patients from the western region of the Black Sea [44].

The increased risk of VT being associated with 677T variant was also reported in Italy by Signorelli *et al.* [45]. In a small Russian study conducted by Shevela *et al.*, the presence of this MTHFR variant increased the risk of VT up to 2-fold, as compared to wild allele [46].

Also, in South America, studies carried out among Chilean and Colombian populations have suggested that C677T polymorphism may be considered as a molecular risk marker for deep vein thrombosis, especially in Chilean patients [47].

A large meta-analysis concerning possible contribution of 28 major polymorphisms in 21 genes to VT development, performed by Gohil *et al.* in 126,525 cases and 184,068 controls from various ethnic groups, has confirmed the clinical relevance of MTHFR 677T allele only in Chinese and Thai populations [48]. However, in case of such significant geographical and ethnic diversity in SNP distribution, the value of a large meta-analysis may be at least questionable.

Contra

In contrast to the reports mentioned above, some studies have negated the association of MTHFR SNPs with development of VT. No such relationship was reported by Ayala *et al.* with regard to the Colombian population [49], as well as to VT patients in Northern Europe. In a Norwegian study by Naess *et al.* the 677TT genotype was present in both, VT and control groups, with the same frequency of 9% and 8%, respectively [50]. Similar observation was confirmed by Simone *et al.* [51], and in a large case-control Dutch study involving 4375 VT patients and 4856 control subjects [52]. Nevertheless, despite no clinically relevant interaction between MTHFR SNPs and other thrombophilia-associated polymorphisms (factor V Leiden and prothrombin G20210A variant) regarding thrombosis occurrence, it has been found that 677T allele significantly increases the VT risk in women using oral contraceptives [51].

CONCLUSIONS

It is noteworthy that factor Leiden and prothrombin G20210A SNP were found in more than 50% of patients with inherited thrombophilia [53], and in approximately 30% and 6% of venous thrombosis-suffering individuals, respectively [7, 54]. However, as mentioned previously, the occurrence of MTHFR SNPs, especially 677T allele, may affect more than 50% of the general population, e.g. in Central Europe. Such a high prevalence of these SNPs cannot be ignored, especially when combined with other pro-thrombotic risk factors – oral contraceptives, immobility, pregnancy, cancer, age, and gender [55-57]. Therefore, we postulate that at least MTHFR C677T SNP

should be included in genetic screening. It seems reasonable especially in cases of recurrent thrombosis, positive family history, or VT episodes in young patients, but also for patients from populations with high prevalence of the mentioned SNP. Furthermore, such screening may be very useful in patients with high risk of hyperhomocysteinemia due to dietary deficiencies – e.g. after gastric surgery, or abnormalities in intestinal absorption. In these cases the MTHFR SNPs-based rationale for intensive folate and B vitamin supplementation would enable more effective anti-thrombotic protection. Thus, although recognised as rather weak single risk factors of venous thrombosis, polymorphisms of MTHFR cannot be underestimated and still require our attention.

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