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Methylenetetrahydrofolate Reductase Gene Polymorphisms and Cardiovascular Diseases

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Abstract

A growing body of evidence suggests that mutations in MTHFR gene are involved in cardiovascular diseases (CVD) - cardiac development, atherosclerosis, myocardial infarction, heart failure, hypertension, aneurysms- and several other disease- cancers, neurological and metabolic disorders. Genetic variations in other genes are added risk for CVD- a leading cause of morbidity and mortality around the globe. Accumulating data over the decade has enhanced our understanding of MTHFR deficiency and diseases associated risk. The frequency of MTHFR 677 C→T and 1298 A→C gene mutations varies substantially in different regions of the world among different racial and ethnic groups. In particular, 677C→T and 1298 A→C variant are associated with clinical manifestation of almost all non-communicable diseases. This review describes the roles of MTHFR gene mutation in CVD and prospective therapies for heart disease treatment.

Keywords: Polymorphism; MTHFR gene; Mutations; Cardiac diseases

Methylenetetrahydrofolate Reductase (*MTHFR*) Gene

Methylenetetrahydrofolate reductase (MTHFR) is a cytosolic enzyme, which contains a non-covalently bound Flavin Adenine Dinucleotide (FAD) cofactor and uses NADPH as the reducing agent. This is an essential enzyme for folate and homocysteine (hcy) metabolisms and exhibits a risk factor for a number of heart diseases [1,2]. MTHFR is responsible for converting the circulating form of folate 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate in multistep processes that converts homocysteine- an amino acid to another amino acid, methionine and S-adenosyl methionine - the common methyl donor for the maintenance of several biological processes (Figure 1). The body uses methionine to make proteins and other important compounds for growth and metabolism. On the other hand, appropriate methylation facilitates the clearance of harmful substances, metabolites and waste products more efficiently.

Over the decade researchers have enhanced our understanding of pathophysiological relation with common and rare MTHFR mutations, enzyme deficiency, elevated hcy and low folate levels in circulation. Of note, it has been reported that compromised MTHFR enzyme activity leads to elevated levels of hcy. Homocysteine is a sulpher containing amino acid, is an oxidant, and play a vital role in oxidation of lipids and lipoproteins, hence augmenting CVD risk [3,4]. Mudd et al. [5] have discovered a severe form of MTHFR enzyme deficiency, which leads to a very serious health conditions- homocysteinuria - in which hcy excretes out in urine. Since the discovery of the role of MTHFR gene mutation in human diseases, this enzyme has received much interest in establishing the association with increased concentration of hcy and heart diseases. There are several case control, retrospective and meta analyses that have demonstrated that MTHFR polymorphism is associated with increased blood hcy concentration and CVD [3,6-9]. The MTHFR 677C→T and 1298 A→C homozygous genotype is associated with premature CAD and other cardiovascular disorders [1,2,10].

On the other hand in the mid-nineties a great piece of discovery - cDNA synthesis - has been published, which paved the way for functional analysis of the MTHFR gene [11]. This transformed the MTFHR research which followed by identification of several rare and common variants including missense variant of alanine to valine at nucleotide 677, which encodes the thermolabile form of the enzyme [3,12].

The mutant TT genotype is linked to elevated circulating hcy levels and the individuals carrying this mutation exhibits low folate levels [13]. 677C→T variant is the most common and prevalent form of MTHFR genetic polymorphisms, which depicts mild to high level of hcy and associated disease manifestation [13-15]. Nonetheless, this variant located in the catalytic domain of the gene and thermolabile in nature affects hcy and folate metabolism. In 1998, another common polymorphism in the MTHFR gene was described, the 1298 A→C transition, which caused an amino acid substitution of glutamate by alanine [16,17]. Sibani et al. [18] reported 33 severe mutation and two common mutations, however Martin et al. [19] reported 65 mutations in MTHFR gene. MTHFR- as a central modulator of folate-hcy-methionine pathway, inspired investigators from all fields to identify and characterize novel mutations in relation to human health. Therefore hundreds (~ 109) of polymorphism - that includes mutations, deletions, duplications, and splicing variants- have been identified [19] and investigations continue to establish the role in CVD risk [20].

Correspondingly, to explore the cause of diseases pathology as a consequence of MHFR gene mutation, scientists developed *Mthfr* knockout mice. They have exhibited a remarkably lower (>60%) enzyme activity in 677C→T variant, resulting in high hcy level among mutant group and also observed high lipid deposition in the major arteries [21]. Cascading effect of hyperhomocysteinemia is the causative factor for high cholesterol deposition in the vessel which initiates atherosclerosis generation and progression, that would lead to myocardial infarction and heart failure. MTHFR gene exists in dimeric form, consisting of 656 amino acid translating a protein that migrates at ~ 74-77 kDa. This is an evolutionary conserved gene throughout organisms from yeast to human. However, mouse depicts the highest homology (>90%). MTHFR gene is located at the short arm of chromosome 1 (1p36.3) and

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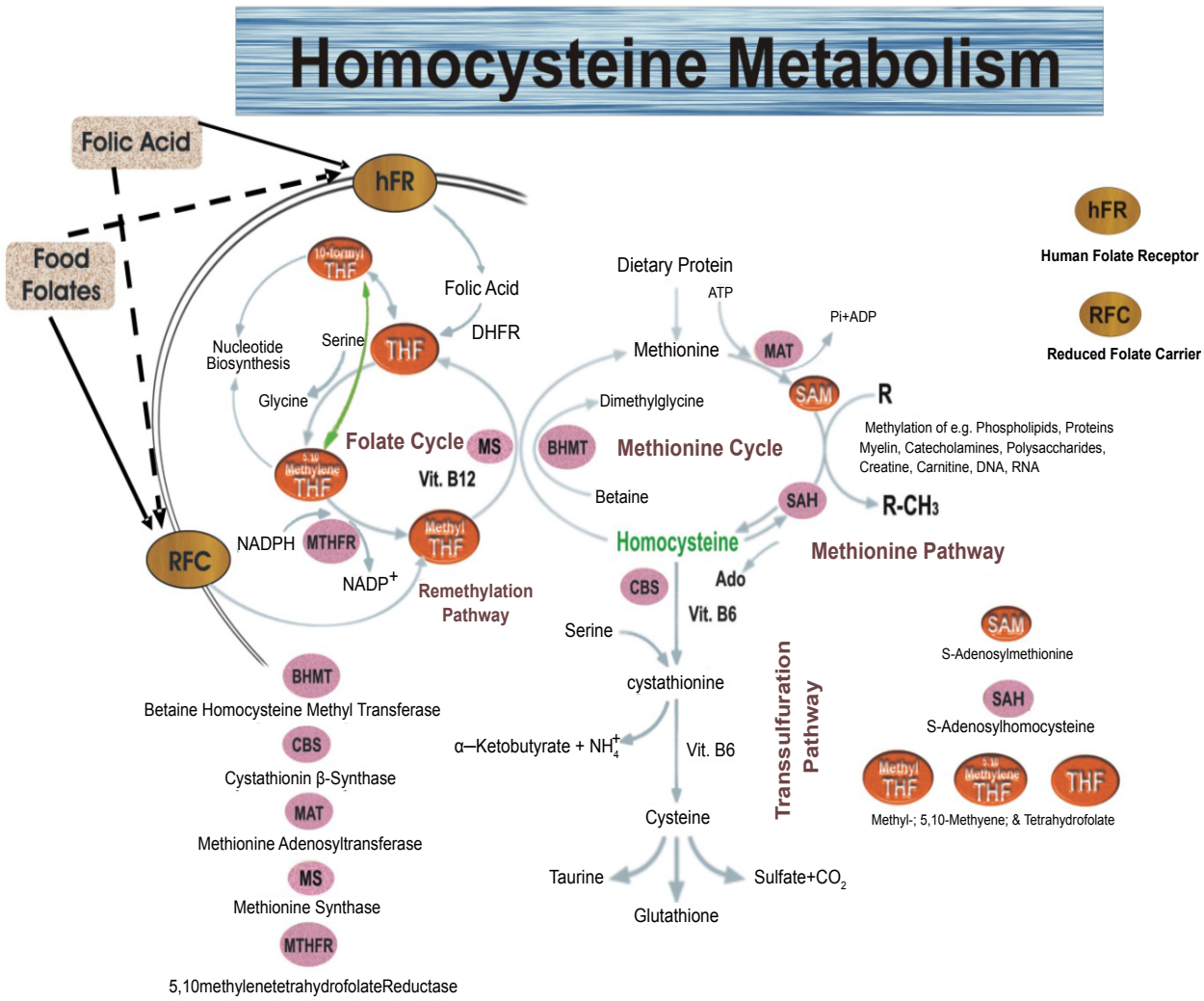

Figure 1: Homocysteine metabolism.

spans ~ 21 Kb. The cDNA sequence is 2.2 kb long and it consists of 11 exons ranging from 102 bp to 432 bp. Intron size ranges from 250 bp to 1.5 kb with one exception of 4.2 kb. Additionally, it has a close physical linkage with CLCN6 [22].

677C→T Mutation and CVD Risk

Indeed CVD is multifactorial disease and causes more than 15 million deaths and huge number of morbid patients. In fact hundreds of genes, environmental and epigenetic factors are involved in CVD manifestation. Correspondingly, mutations in atherogenic lipid and lipoprotein genes are responsible for high cholesterol levels in CVD. Thus it is true with MTHFR 677C→T gene mutation and increased hcy level in CVD. Moreover the findings of meta-statistical analyses reported significant link between hcy levels and MHTFR genotypes [23].

MTHFR, a gene involved in the metabolism of Hcy, is of particular medical interest as being a versatile member of the genetic risk factors for many diseases. Of note, numerous results have been published showing augmented risk of CVD and TT genotype. Therefore, the finding of an increased risk of CVD associated with MTHFR is not surprising because TT genotype is a strong genetic determinant for hyperhomocysteinemia. Similar observation was reported that 677TT genotype could be an inherited risk factor for heart disease [15,24,25].

Additionally many previous studies have demonstrated mild to high risk of CVD development and progression or no effect at all [26-30]. This discrepancy could be due to different selection criteria, different methods for hcy and folate measurements. The finding of the studies could be different as some countries have folic acid supplementation in their food habits that may cause lower hcy levels and reduced CVD risk [31].

Large meta-analyses were conducted on individual data from case–control studies relating the 677C→T MTHFR genotype and CVD. In one report, 40 studies comprising 11,162 patients with CAD were selected, whereas in the other report 120 studies were considered comprising 19,993 patients with different forms of vascular disease (CVD, DVT and stroke). Consistently, both studies concluded that subjects with the MTHFR 677TT genotype have modest but statistically significant increased risk of CVD when compared with those bearing the wild-type genotype [4,32]. In view of that, another meta-analysis consisting of more than 20,000 subjects determined an increased CVD risk of subjects carrying 677TT MTHFR genotype.

This supports the underlying link of 677TT genotype with increased hcy and CVD [33-35].

677T Allele and Genotypes

Involvement of MTHFR in hcy-methionine cycle makes it an important player to investigate the causal 'T' allele/genotype frequency in CVD. The prevalence of MTHFR 677C→T gene polymorphism varies substantially with ethnic and racial groups worldwide. A diverse nature of 'T' allele incidence ranging from 59.0% in Mexicans, 44.9% in Brazilians, 33.3 to 43.8% in South European countries, 37.0 to 42.0% in Japanese, 33.0 to 38.0% in Chinese 38.0% in Canadian, 32.2% in Americans, 20% to 33.3% in Middle East Asia, 20.8% in Asians, 18.6% in European, 6.6% in Africans, and 4.7% in Australian [2,3,13,27,35,36].

A few studies reported from India and Indian subcontinent [24,37], have shown that mutant 'T' allele prevalence varies greatly across India, which is similar to the pattern reported in Chinese populations [36]. In our previous study, we found that the 'T' allele frequency was 17.0% [24], which is very similar to previous results (18.4%) from our centre [38]. However, there is a wide variation between north Indian (14.5-18.4%) and south Indians (9.0%) and a very low prevalence in Sri Lankans (4.9%) [39].

In the same way, MTHFR 677TT genotype in CVD subjects is ~ 7.9 %, which is higher than the general populations. It means that TT genotype may be the risk factor of heart disease [24]. The TT genotype frequency in Caucasians, Chinese and Japanese populations is 10-16% [35,40]. The precise reasons underlying the relatively low prevalence among Indians are not known.

Overall the frequency of T allele and mutant TT genotype are highly diverse in the population, so their risk in disease development also varies significantly dependent on the ethnic, race and food habits.

1298A→C Mutation and CVD Risk

Indeed, another most common MTHFR genetic polymorphism 1298A→C, which change glutamate to alanine at the 1298 nucleotide. This variant resides at exon 7 in the regulatory domain, while 677 C→T variant resides at exon 4 in the catalytic domain. It plays a critical role in various diseases including CVD. The effect of enzymatic activity of 1298A→C changes is lesser than 677 C→T change, which is an ~35 % decrease, meaning that it retains 65% of the total enzymatic activity.

On the other hand, 1298A→C variant has been less studied than 677 C→T variant. Moreover, the effect of 1298 A→C of hcy and folate level is milder, therefore the disease risk severity is less than 677 C→T variant [27,39-42]. Subsequently, a few studies did not find increase in hcy levels among homozygous (1298CC) even though they had decreased enzymatic activity, which may be due to non-thermolabile nature [16,17].

Furthermore, investigators dissect the effect of these two common variants at molecular levels using recombinant technology. They demonstrated how FAD binds and dissociates from the enzyme complex during the multistep metabolic pathways. It also help in determining metabolites, cofactors which is very important in MTHFR enzyme activity [43,44].

1298 C Allele and Genotypes

The 'C' allele frequency also varies greatly across the globe. Canadians and Europeans (36%), Israelites (34%), Americans (32%) and Portuguese (28.3%), and higher compared to Chinese (17%) [36] and Africans (21%) [41]. The 'C' allele frequency in Indians varies geographically and ethnically. South Indians represents higher prevalence (36%) [39], compared to North Indians 10-23% [24].

Similarly, the mutant (1298 CC) genotype frequency is ethnic and race dependent. The prevalence in Caucasians is ~ 10% [41,45], Arabians (9.1%) [29], while in Indians it ranges from 3.0% to 17.8% with varying amount of disease severity [24,39,42].

Conclusion

Having variant MTHFR allele and elevated hcy levels is an indicator of CVD risk and severity. MTHFR 677C→T and MTHFR 1298 A→C are two common polymorphisms that are associated with increased risk for many diseases due to the impaired enzymatic activity of the protein encoded by the minor alleles. Determination of MTHFR status for either 677 C→T or 1298 A→C has predictive value. Indeed MTHFR variations appear to be medically irrelevant, if an individual's homocysteine level is normal. Of note, recent studies indicate that lowering an elevated homocysteine level may decrease the risk of atherosclerosis and CVD. Further study to explore the association at the molecular level and folic acid supplementation in lowering blood hcy levels is warranted, since increased folate levels are beneficial for a number health conditions.

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