Methylenetetrahydrofolate reductase

Methylene tetrahydrofolate reductase (**MTHFR**) is the rate-limiting enzyme in the methyl cycle, and it is encoded by the *MTHFR* gene.[1] Methylenetetrahydrofolate reductase catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a cosubstrate for homocysteine remethylation to methionine. Natural variation in this gene is common in healthy people. Although some variants have been reported to influence susceptibility to occlusive vascular disease, neural tube defects, Alzheimer's disease and other forms of dementia, colon cancer, and acute leukemia, findings from small early studies have not been reproduced. Some mutations in this gene are associated with methylene tetrahydrofolate reductase deficiency.[2][3][4]

1 Biochemistry

In the rate-limiting step of the methyl cycle, MTHFR irreversibly reduces 5,10-methylenetetrahydrofolate (substrate) to 5-methyltetrahydrofolate (product).

- 5,10-methylene tetrahydrofolate is used to convert dUMP to dTMP for *de novo* thymidine synthesis.
- 5-Methyltetrahydrofolate is used to convert homocysteine (a potentially toxic amino acid) to methionine by the enzyme methionine synthase. (Note that homocysteine can also be converted to methionine by the folate-independent enzyme betaine-homocysteine methyltransferase (BHMT))

MTHFR contains a bound flavin cofactor and uses NAD(P)H as the reducing agent.

2 Structure

Mammalian MTHFR is composed of an N-terminal catalytic domain and a C-terminal regulatory domain. MTHFR has at least two promoters and two isoforms (70 kDa and 77 kDa).[5]

3 Regulation

MTHFR activity may be inhibited by binding of dihydrofolate (DHF)[6] and S-adenosylmethionine (SAM, or AdoMet).[7] MTHFR can also be phosphorylated - this decreases its activity by ~20% and allows it to be more easily inhibited by SAM.[8]

4 Genetics

The enzyme is coded by the gene with the symbol *MTHFR* on chromosome 1 location p36.3 in humans.[9] There are DNA sequence variants (genetic polymorphisms) associated with this gene. In 2000 a report brought the number of polymorphisms up to 24.[10] Two of the most investigated are C677T (rs1801133) and A1298C (rs1801131) single nucleotide polymorphisms (SNP).

4.1 C677T SNP (Ala^{222}Val)

Main article: rs1801133

The MTHFR nucleotide at position 677 in the gene has two possibilities: C (cytosine) or T (thymine). C at position 677 (leading to an alanine at amino acid 222) is the normal allele. The 677T allele (leading to a valine substitution at amino acid 222) encodes a thermolabile enzyme with reduced activity.

Individuals with two copies of 677C (677CC) have the most common genotype. 677TT individuals (homozygous) have lower MTHFR activity than CC or CT (heterozygous) individuals. About ten percent of the North American population are T-homozygous for this polymorphism. There is ethnic variability in the frequency of the T allele – frequency in Mediterranean/Hispanics is greater than the frequency in Caucasians which, in turn, is greater than in Africans/African-Americans.[11]

The degree of enzyme thermolability (assessed as residual activity after heat inactivation) is much greater in 677TT individuals (18-22%) compared with 677CT (56%) and 677CC (66-67%).[12] Individuals of 677TT are predisposed to mild hyperhomocysteinemia (high blood homocysteine levels), because they have less active MTHFR available to produce 5-methyltetrahydrofolate (which is used to decrease homocysteine). Low dietary intake of the vitamin folic acid can also cause mild hyperhomocysteinemia.

Low folate intake affects individuals with the 677TT genotype to a greater extent than those with the 677CC/CT genotypes. 677TT (but not 677CC/CT) in-

dividuals with lower plasma folate levels are at risk for elevated plasma homocysteine levels.[13] In studies of human recombinant MTHFR, the protein encoded by 677T loses its FAD cofactor three times faster than the wild-type protein.[14] 5-Methyl-THF slows the rate of FAD release in both the wild-type and mutant enzymes, although it is to a much greater extent in the mutant enzyme.<ref name="YamadaK2001Effects"/ Low folate status with the consequent loss of FAD enhances the thermolability of the enzyme, thus providing an explanation for the normalised homocysteine and DNA methylation levels in folate-replete 677TT individuals.

This polymorphism and mild hyperhomocysteinemia are associated with neural tube defects in offspring, increased risk for [http://www.rbej.com/content/2/1/65 complications of pregnancy other complications of pregnancy, arterial and venous thrombosis, and cardiovascular disease.[15] 677TT individuals are at an increased risk for acute lymphoblastic leukemia[16] and colon cancer.[17]

Mutations in the *MTHFR* gene could be one of the factors leading to increased risk of developing schizophrenia.[18] Schizophrenic patients having the risk allele (T\T) show more deficiencies in executive function tasks.[19]

The C677T genotype is associated with increased risk of recurrent pregnancy loss (RPL) in non Caucasians.[20]

There is also a tentative link between MTHFR mutations and dementia. One study of an elderly Japanese population[21] found correlations between the MTHFR 677CT mutation, an Apo E polymorphism, and certain types of senile dementia. Other research has found that individuals with folate-related mutations can still have a functional deficiency even when blood levels of folate are within the normal range,[22] and recommended supplementation of methyltetrahydrofolate to potentially prevent and treat dementia (along with depression). A 2011 study[23] from China also found that the C677T SNP was associated with Alzheimer's disease in Asian populations (though not in Caucasians).

4.2 A1298C SNP (Glu^{429}Ala)

At nucleotide 1298 of the MTHFR, there are two possibilities: A or C. 1298A (leading to a Glu at amino acid 429) is the most common while 1298C (leading to an Ala substitution at amino acid 429) is less common. 1298AA is the "normal" homozygous, 1298AC the heterozygous, and 1298CC the homozygous for the "variant". In studies of human recombinant MTHFR, the protein encoded by 1298C cannot be distinguished from 1298A in terms of activity, thermolability, FAD release, or the protective effect of 5-methyl-THF.[14] The C mutation does not appear to affect the MTHFR protein. It does not result in thermolabile MTHFR and does not appear to affect homocysteine levels. It does, however, affect the conversion of MTHF to BH4 (tetrahydrobiopterin), an important cofactor in the production of neurotransmitters, synthesis of nitric oxide, and detoxification of ammonia.

There has been some commentary on a 'reverse reaction' in which tetrahydrobiopterin (BH4) is produced when 5-methyltetrahydrofolate is converted back into methylenetetrahydrofolate. This however is not universally agreed upon. That reaction is thought to require 5-MTHF and SAMe. An alternative opinion is that 5-MTHF processes peroxynitrite, thereby preserving existing BH4, and that no such 'reverse reaction' occurs.

4.3 Detection of MTHFR polymorphisms

A triplex tetra-primer ARMS-PCR method was developed for the simultaneous detection of C677T and A1298C polymorphisms with the A66G MTRR polymorphism in a single PCR reaction.[24]

4.4 Severe MTHFR deficiency

Severe MTHFR deficiency is rare (about 50 cases worldwide) and caused by mutations resulting in 0-20% residual enzyme activity.[10] Patients exhibit developmental delay, motor and gait dysfunction, seizures, and neurological impairment and have extremely high levels of homocysteine in their plasma and urine as well as low to normal plasma methionine levels.

5 As a drug target

Inhibitors of MTHFR and antisense knockdown of the expression of the enzyme have been proposed as treatments for cancer.[25] The active form of folate, L-methylfolate, may be appropriate to target for conditions affected by MTHFR polymorphisms.[26]

6 Reaction and metabolism

The overall reaction catalyzed by MTHFR is illustrated on the right. The reaction uses an NAD(P)H hydride donor and an FAD cofactor. The *E. coli* enzyme has a strong preference for the NADH donor, whereas the mammalian enzyme is specific to NADPH.

Click on genes, proteins and metabolites below to link to respective articles. [§ 1]

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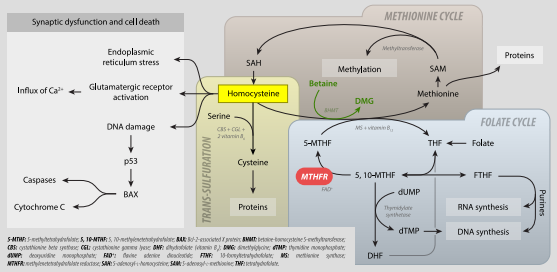

MTHFR metabolism: folate cycle, methionine cycle, trans-sulfuration and hyperhomocysteinemia. 5-MTHF: 5-methyltetrahydrofolate; 5,10-methyltetrahydrofolate; BAX: Bcl-2-associated X protein; BHMT: betaine-homocysteine S-methyltransferase; CBS: cystathionine beta synthase; CGL: cystathionine gamma-lyase; DHF: dihydrofolate (vitamin B9); DMG: dimethylglycine; dTMP: thymidine monophosphate; dUMP: deoxyuridine monophosphate; FAD+ flavine adenine dicucleotide; FTHF: 10-formyltetrahydrofolate; MS: methionine synthase; MTHFR: mehtylenetetrahydrofolate reductase; SAH: S-adenosyl-L-homocysteine; SAME: S-adenosyl-L-methionine; THF: tetrahydrofolate.

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Fluorouracil (5-FU) Activity edit

[1] The interactive pathway map can be edited at WikiPathways: "FluoropyrimidineActivity_WP1601".

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