David S. Rosenblatt

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CLINICALLY IMPORTANT GENES

Methylenetetrahydrofolate reductase

David S. Rosenblatt, MD

Dr. Rosenblatt is Director, Division of Medical Genetics, Department of Medicine, McGill University Health Centre, Montreal, Que.

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Introduction

These are exciting times for those interested in the interaction of genetic and environmental factors in disease. Folic acid has recently gained a great deal of prominence as a biologically important molecule. The ingestion of folate before conception and continued during pregnancy can protect against both the occurrence and the recurrence of birth defects, in particular neural tube defects such as anencephaly and spina bifida.[1] In addition, in recent years, homocysteine has been shown to be an independent risk factor for cerebrovascular, coronary artery and thrombotic diseases; folate is an effective way to lower levels of total plasma homocysteine (tHcy).[2] A role for folate has also been proposed in the etiology and prevention of a wide variety of diseases including cancer and Alzheimer disease.[3-5]

Biochemistry

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folic acid metabolism. This brief review summarizes the impact of a common polymorphism in MTHFR on the occurrence of these various conditions, on the dietary requirement for folate and some interactions with other vitamins of the B group.

MTHFR is the enzyme responsible for the reduction of methylenetetrahydrofolate, which is a key single carbon donor required for thymidine synthesis, to methyltetrahydrofolate, which is essential for homocysteine remethylation to methionine. MTHFR is thought to be irreversible in human cells; the only way for folate to re-enter the tetrahydrofolate pool is by way of the vitamin B_{12}-dependent methionine synthase reaction. The interconnection of the enzymes involved in folate metabolism is shown in Fig. 1.

Methylenetetrahydrofolate reductase deficiency and genetic polymorphisms

Early studies on some patients with severe MTHFR deficiency showed that the residual specific activity of the enzyme was not stable to heat.[6] Subsequent studies showed that in some families of such patients, parents showed intermediate levels of enzyme activity and in some cases a heat-labile MTHFR.[7] Studies on the heat stability of MTHFR in patients with heart disease showed that there was an increased incidence of heat-labile MTHFR among such patients.[8]

With the cloning of the gene for MTHFR, it soon became evident that the reason for a thermolabile enzyme in the general population was the presence of a common polymorphism (677C→T) resulting in the change of an alanine to a valine. This polymorphism has an allele frequency of about 30% in the population and is associated with a two-thirds decrease in MTHFR-specific activity, as well as an enzyme that is not stable to heat.[9,10] This polymorphism has also been found, in combination with severe mutations,

in patients with the severe form of MTHFR deficiency, and presumably is responsible for the thermolability seen in those patients.[11] A second polymorphism, 1298A→C, which is also common, changing a glutamate to an alanine in the region of MTHFR that binds adenosylmethionine, is also associated with decreased MTHFR-specific activity but not with enzyme lability.[12,13]

Because of the interconnection of the enzymes involved in folate metabolism it is possible to interpret the effect of alterations in the activity of MTHFR in different ways. For example, MTHFR activity is decreased in individuals with the 677C→T polymorphism. This would result in a relative lack of the product, methyltetrahydrofolate, and thus decreased conversion of homocysteine to methionine, and less availability of methyl groups for methylation reactions that require adenosylmethionine. On the other hand, levels of methylenetetrahydrofolate would be expected to be elevated in MTHFR deficiency, providing more one-carbon groups for thymidylate synthesis.

Patients with a deficiency in MTHFR due to a severe mutation in the gene on chromosome 1, have elevated blood and urinary levels of homocysteine. Because there is a decreased source of methyl donors for methionine formation, these patients have decreased blood levels of methionine. Because there is no shortage of methylenetetrahydrofolate, the substrate for MTHFR, patients do not have megaloblastic anemia. To date there are only about 50 patients known with severe MTHFR deficiency, and causal mutations have been found in about half of them.[14] Although most patients have been clinically affected in childhood with developmental delay, some have only presented later in life with thrombosis or neurologic disease. Some have even been completely free of symptoms.[15]

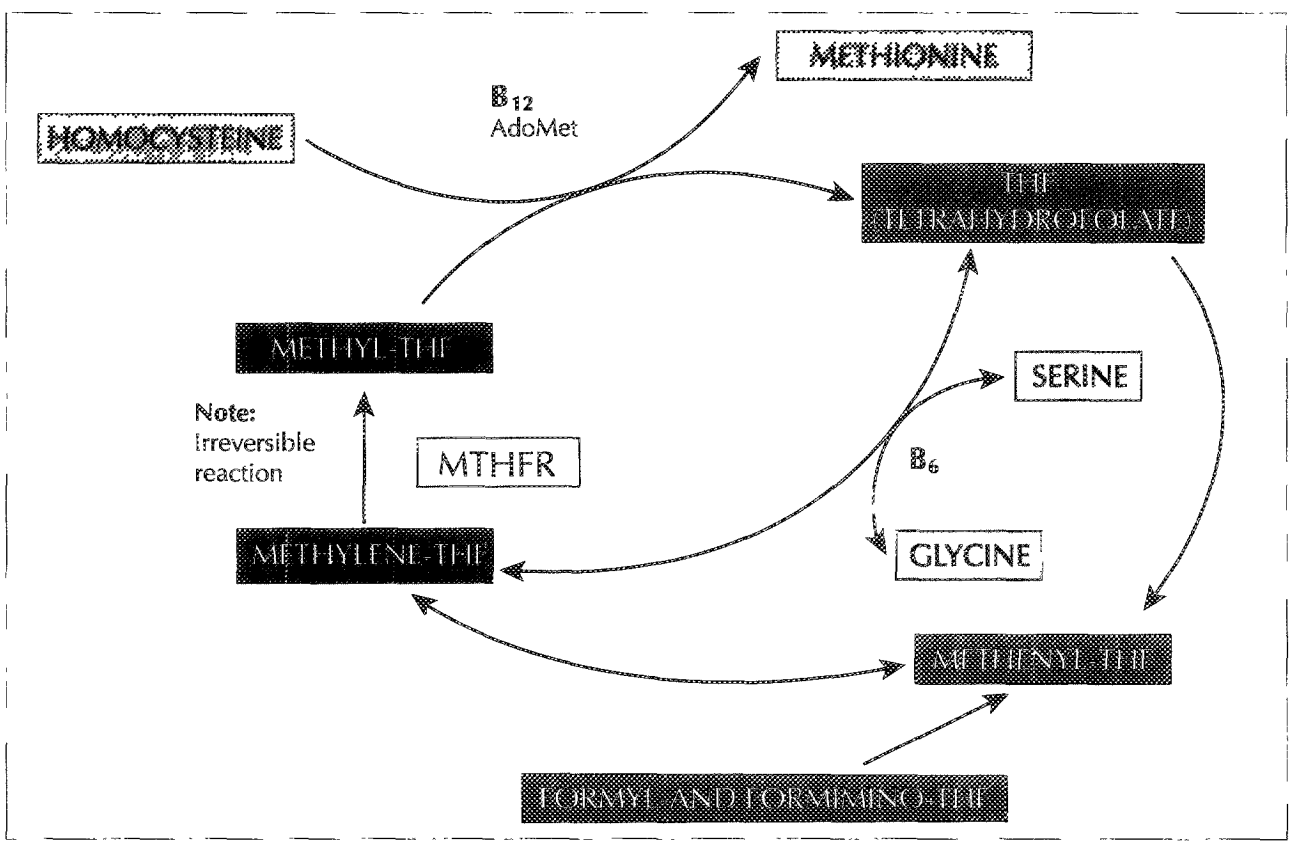

Fig. 1: Interrelationships of some intermediates of folate metabolism. Folic acid (folate) is not a natural substance; folate and dihydrofolate are reduced to enter the tetrahydrofolate (THF) pool. Formyl THF is formed when THF accepts a 1-C fragment as formate; formimino THF is generated when histidine is catabolized. Methylenetetrahydrofolate reductase (MTHFR) provides 1-C fragments for methionine synthesis from homocysteine, a reaction requiring the cofactors vitamin B_{12} and adenosylmethionine (AdoMet).

Since it has been shown that the effect of the 677C→T polymorphism can be modified by folate nutritional status, studies in North America are now complicated by the fortification of cereal grains with folic acid.[16] This has changed the baseline population levels for homocysteine and may affect clinical trials to examine the effect of multivitamins on the incidence of vascular disease.

Clinical significance

What does all this mean for the clinician? When studies on a thermolabile MTHFR required the collection of large numbers of leukocytes for the determination of enzyme activity before and after heating, there were only a small number of studies that attempted to look at the correlation between MTHFR and common disease. All this has changed with the cloning of the gene and the discovery that the cause of the heat lability in the general population was the 677C→T polymorphism. This has meant that population studies could easily be done using small amounts of stable DNA. The range of disorders that have now been studied are vast and include birth defects, Down syndrome, preeclampsia, vascular disease, Alzheimer disease, colon cancer, leukemia, diabetes and many others.[2,3,17-19]

In looking at these studies it is important to note that the frequency of the 677C→T polymorphism in MTHFR varies among different ethnic groups and is particularly low in blacks.[20] Thus, it is essential that studies to look at differences in cases and controls use ethnically matched groups.

There is now good evidence to suggest that elevated tHcy is an independent risk factor for vascular disease.[2] The earliest studies showing an effect of a thermolabile MTHFR in heart disease were difficult to interpret because the thermolabile enzyme was not always associated with elevated homocysteine levels. This has been clarified recently with the finding that the 677C→T polymorphism is associated with elevated tHcy levels only in patients who are relatively deficient in folic acid.[21] Thus, it is not the polymorphism itself that constitutes a risk, but the effect of the polymorphism on homocysteine levels in patients who have borderline folate status.

The 677C→T polymorphism in MTHFR is a genetic risk factor for neural tube defects, particularly in the presence of folate insufficiency.[22] There is good evidence that this polymorphism is also associated with elevated tHcy levels, which in themselves are associated with vascular disease.[2] There is no good evidence that the polymorphism itself is associated with the risk of vascular disease.[23] There is some evidence that the 677C→T polymorphism may be protective for colon cancer[3] and leukemia,[19] but additional studies are needed to confirm these findings.

No one genetic or environmental factor operates in isolation. The metabolism of folate and vitamin B_{12} (cobalamin) intersect at the methionine synthase reaction that requires forms of both vitamins as cofactors. Recently the genes for methionine synthase (MTR) and methionine synthase reductase (MTRR) have been cloned. Polymorphisms have been described in both of the genes, and studies have begun looking at the effect of these polymorphisms, both alone and in combination, on many of the diseases for which an affect has been postulated for the 677C→T polymorphism in MTHFR.

How does the clinician use the present information? Several university and commercial laboratories are offering the 677C→T polymorphism in MTHFR as a clinical test. As part of the investigation of recurrent neural tube defect or of hyperhomocysteinemia, it is not unreasonable to document the presence or absence of the polymorphism. However, the polymorphism is not a good predictor of an individual's risk for a particular disease and should not be used as the complete explanation for a phenotype in any individual patient.

If methylation reactions are involved in birth defects and if elevated homocysteine is a risk factor for vascular disease, the polymorphism may be considered detrimental. On the other hand, if it is desirable to have relatively higher levels of methylenetetrahydrofolate, the 677C→T polymorphism might be considered to be beneficial. As it is difficult to predict in advance in which clinical situations the relative balance of remethylation and endogenous purine and pyrimidine synthesis assume more importance, it becomes necessary to look empirically at the effect of the polymorphism in many different clinical situations.

With the completion of the human genome project, many more genes and many more polymorphisms

will be identified. The challenge will be how to integrate this information into clinical judgement and how to use partial information before the story is complete.

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Reprint requests to: Dr. David S. Rosenblatt, Director, Division of Medical Genetics, Department of Medicine, McGill University Health Centre, Room H5-63, 687 Pine Ave. W, Montreal QC H3A 1A1; fax 514 843-1473