PREFACE

Methylenetetrahydrofolate reductase (MTHFR) is a critical enzyme in both folate and homocysteine metabolism. It first achieved medical recognition in 1972, with the report of severe deficiency of MTHFR in a patient with homocystinuria, an inborn error of metabolism characterized by marked elevation of homocyst(e)ine in plasma and urine. Although the majority of cases of homocystinuria are due to a deficiency of the first enzyme in the transsulfuration pathway for homocysteine metabolism, cystathionine-β-synthase (CBS), disruption of homocysteine remethylation to methionine can also result in homocystinuria. With the identification of additional patients with severe MTHFR deficiency, the heterogeneity of this disorder became manifest. Of particular relevance to the comments below was the report of a heat-sensitive MTHFR in some homocystinuric patients, which was assumed to be caused by a deleterious mutation. A comprehensive discussion of severe MTHFR deficiency can be found in Chapter 4 of this book.

Patients with homocystinuria, due to transsulfuration or remethylation defects, frequently suffer from thromboses and display arteriosclerotic occlusive changes in their vasculature. These types of observations led to the hypothesis that more moderate elevations in plasma homocysteine could contribute to the risk for cardiovascular disease. In 1988, a thermolabile form of MTHFR was identified in a group of American patients with coronary artery disease, following enzymatic assays in lymphocyte extracts that had been heated at 46°C. for 5 minutes. This heat-sensitive enzyme appeared to be more common in the patient group compared to the control group and was associated with a relatively milder deficiency than that observed in patients with homocystinuria. The identification of some homocystinuric families with both the severe and mild deficiency further complicated the situation. Studies of MTHFR were limited at this time, since only a few laboratories, those with biochemical expertise, could routinely measure MTHFR activity.

In the 1990s, molecular genetic investigations of MTHFR were undertaken in an attempt to clone the cDNA/gene and to identify the molecular basis of severe and mild MTHFR deficiency. In 1994, the isolation of the cDNA was reported, with identification of the first mutations in severe MTHFR deficiency in homocystinuric patients. This report was quickly followed by identification of a common variant, a missense mutation at bp 677, which encoded the thermolabile enzyme and predisposed

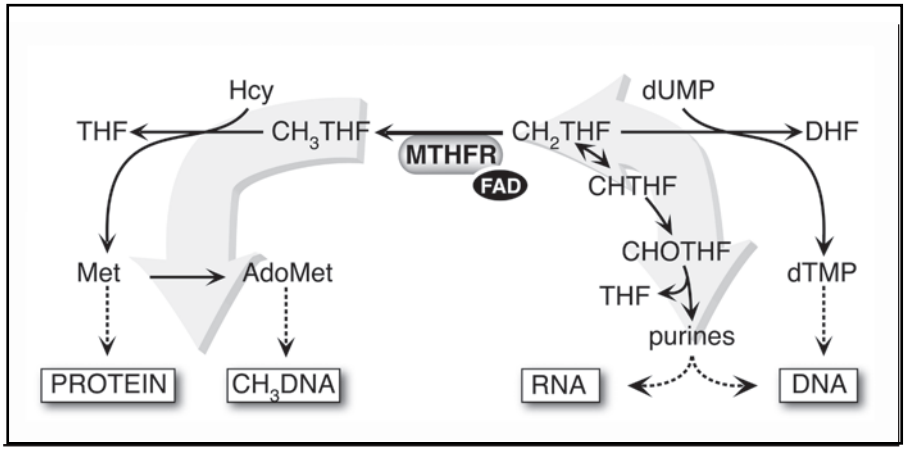

Figure 1. Methylenetetrahydrofolate reductase and folate distribution. Methylenetetrahydrofolate reductase (MTHFR) catalyzes the irreversible reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. It affects the distribution between folate species used for DNA and RNA syntheses and the 5-methyltetrahydrofolate form required for homocysteine remethylation to methionine and subsequent protein synthesis and DNA methylation. AdoMet= S-adenosylmethionine; CH3THF= 5-methyltetrahydrofolate; CH2THF= 5,10-methylenetetrahydrofolate; CHTHF= methenyltetrahydrofolate; CHO= formyltetrahydrofolate; CH3DNA= methylated DNA; DHF= dihydrofolate; Hcy= homocysteine; Met= methionine; THF= tetrahydrofolate.

to mild or moderate hyperhomocysteinemia. Subsequent studies demonstrated that the thermolability of MTHFR in some patients with homocystinuria was due to the presence of the common variant, in addition to the presence of the 2 deleterious mutations that contributed to the severe deficiency and homocystinuria.

MTHFR is a FAD-dependent enzyme which catalyzes the irreversible conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydofolate. 5-Methyltetrahydofolate in turn serves as a methyl donor in the remethylation of homocysteine (Hcy) to methionine. The enzyme therefore resides at an important metabolic branch point directing the folate pool towards Hcy remethylation and DNA methylation at the expense of DNA and RNA biosynthesis (Fig. 1). This explains why individuals with the thermolabile variant are predisposed to elevated plasma homocysteine under conditions of impaired folate status; low riboflavin may also influence this association since riboflavin is the precursor for FAD. MTHFR deficiency influences methylation of DNA and possibly other methyl

acceptors, and may affect the levels of nucleotide pools available for DNA synthesis. These metabolic changes presumably contribute mechanistically to some of the disease associations observed with the MTHFR 677C→T polymorphism.

Mutation, polymorphism, variant and sequence change—these terms are used interchangeably throughout the book to denote the 677C→T nucleotide substitution. A mutation refers to any nucleotide change; a mutation can be benign or deleterious. A polymorphism is simply a mutation that is common in the population (>1% prevalence); similarly, a polymorphism can be benign or deleterious. All the above terminologies are therefore correct when discussing the substitution at bp 677.

The molecular genetic assay for the polymorphism at bp 677 was straightforward. It The polymorphism was common in virtually all populations. A growing list of phenotypes appeared to be influenced by homocysteine or folate levels (birth defects, pregnancy complications, psychiatric disorders, etc.). These 3 facts laid the foundation for a surge in the number of reports on MTHFR. Figure 2 depicts the number of publications per year on MTHFR, showing the rapid rise in articles following the reports of the cDNA sequence and of the polymorphism in 1994 and 1995, respectively.

Investigations of MTHFR deficiencies have paralleled the transition in medical genetics from isolation of genes involved in single gene disorders to identification of variants involved in complex multifactorial diseases. The advent of molecular genetic technologies in the 1970s and 1980s first resulted in the identification of genes involved in "classic" genetic disorders such as the hemoglobinopathies, Duchenne muscular dystrophy and cystic fibrosis, to name a few; the homocystinurias would also fall into this category. In the new millennium, following the completion of the sequencing of the human genome, major efforts are being devoted to identification of single nucleotide polymorphisms (SNPs) that influence predisposition or risk for complex traits. MTHFR serves as an interesting model for both types of disorders.

Predisposition or risk for disease implies that a genetic variant on its own is unlikely to cause disease; other genetic variants and nongenetic factors are required for manifestation of the phenotype. Investigations on MTHFR have been quite instructive in this regard, since there is a limited number of polymorphisms in the human genome that clearly influence risk for complex traits. It has become evident that the single 677C→T variant may only contribute to a modest increase in risk; other genetic variants and nongenetic factors (folate status, riboflavin status, medication, or age) can modify the risk contributed by the variant.

This book covers many of the complex traits that have been reported to be influenced by the well-characterized 677C→T variant; there is less information

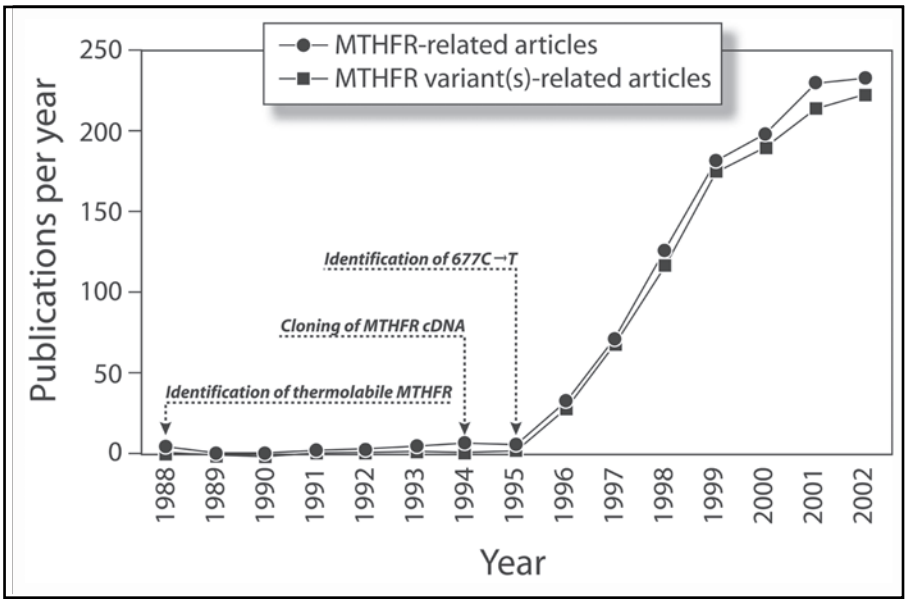

Figure 2. Publications on methylenetetrahydrofolate reductase (MTHFR) from 1988 to 2002. Publications on MTHFR were identified by literature searches of MEDLINE with the keywords "MTHFR" or "methylenetetrahydrofolate reductase" (Filled circles). To identify publications that discussed MTHFR and mutations/polymorphisms, MEDLINE was searched for (MTHFR or methylenetetrahydrofolate reductase) AND (polymorphism* OR 677 OR C677T OR variant OR mutation OR genotype OR risk factor OR thermolabile) (Filled squares). Figure was prepared by Dr. Daniel Leclerc, McGill University.

on the 1298A→C variant, but it is discussed where appropriate. It is quite surprising, and unique, that a single variant should influence such a wide variety of clinical conditions. However, given the critical role of folates in DNA synthesis and repair, in homocysteine regulation, and in the methylation cycle, it is understandable why the interest curve in MTHFR (Fig. 2) is currently at 200–250 publications per year.

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