Update and new concepts in vitamin responsive disorders of folate transport and metabolism

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Abstract Derivatives of folic acid are involved in transfer of one-carbon units in cellular metabolism, playing a role in synthesis of purines and thymidylate and in the remethylation of homocysteine to form methionine. Five inborn errors affecting folate transport and metabolism have been well studied: hereditary folate malabsorption, caused by mutations in the gene encoding the proton-coupled folate transporter (SLC46A1); glutamate formiminotransferase deficiency, caused by mutations in the FTCD gene; methylenetetrahydrofolate reductase deficiency, caused by mutations in the MTHFR gene; and functional methionine synthase deficiency, either as the result of mutations affecting methionine synthase itself (cblG, caused by mutations in the MTR gene) or affecting the accessory protein methionine synthase reductase (cblE, caused by mutations in the MTRR gene). Recently additional inborn errors have been identified. Cerebral folate deficiency is a clinically heterogeneous disorder, which in a few families is caused by mutations in the FOLR1 gene. Dihydrofolate reductase deficiency is characterized by megaloblastic anemia and cerebral folate deficiency, with variable neurological findings. It is caused by mutations in the DHFR gene. Deficiency in the trifunctional enzyme containing methylenetetrahydrofolate dehydrogenase, methenyltetrahydrofolate cyclohydrolase and formyltetrahydrofolate synthetase activities, has been identified in a single patient with megaloblastic anemia, atypical hemolytic uremic syndrome and severe combined immune deficiency. It is caused by mutations in the MTHFD1 gene.

Abbreviations

DHFR Dihydrofolate reductase
FormiminoTHF Formiminotetrahydrofolate
FormylTHF Formyltetrahydrofolate
FRα FRβ Folate receptors alpha and beta
MethenylTHF Methenyltetrahydrofolate
MethylTHF Methyltetrahydrofolate
MethyleneTHF Methylenetetrahydrofolate
MTHFR Methylene tetrahydrofolate reductase
PCFT Proton-coupled folate transporter
RFC Reduced folate carrier
THF Tetrahydrofolate

Derivatives of folic acid play a critical role in cellular one-carbon metabolism, acting as donors and recipients of one-carbon units involved in synthesis and breakdown of amino acids (serine, glycine, methionine and homocysteine, and histidine) and in synthesis of thymidine and purines. Folic acid consists of a pteroic acid moiety with a bound glutamate residue. Biologically active folic acid derivatives are generally 5,6,7,8-tetrahydrofolates with a one-carbon unit bound to nitrogen 5, to nitrogen 10, or forming a bridge between the two nitrogen atoms. There is also a

References to electronic databases: Online Mendelian Inheritance in Man http://omim.org

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chain of glutamate residues attached to the initial residue by γ-peptide bonds, so that biological folates are one-carbon substituted tetrahydrofolate polyglutamates. Polyglutamate forms typically have decreased $K_m$ for folate dependent enzymes compared to the equivalent monoglutamate form, and are efficiently channeled between active sites of multifunctional proteins. In addition, folate polyglutamates are not transported across the mitochondrial membrane, resulting in the presence of separate cytoplasmic and mitochondrial folate pools, with consequences for the compartmentalization of cellular folate metabolism.

Figure 1 shows the interrelationships of cellular tetrahydrofolate (THF) derivatives and the sources and uses of one-carbon units. One-carbon units are derived primarily from serine and glycine, by serine hydroxymethyltransferase and the glycine cleavage system, respectively, with histidine serving as a potential source in some tissues. 10-formylTHF is required for two separate steps in de novo purine biosynthesis, contributing carbons 2 and 8 of the purine ring. 5,10-methyleneTHF is required for conversion of deoxyuridylate to thymidylate, catalyzed by thymidylate synthase. 5-methylTHF provides the one-carbon unit in the cobalamin-dependent methylation of homocysteine to form methionine, catalyzed by methionine synthase. Each of these reactions generates THF except for the thymidylate synthase reaction, which results in formation of dihydrofolate. Regeneration of THF depends on activity of dihydrofolate reductase.

Interconversion of one-carbon substituted THF derivatives is catalyzed by separate enzymes in the cytoplasm and mitochondria. In the cytoplasm, interconversion of 5,10-methyleneTHF and 5,10-methenylTHF (methylenTHF dehydrogenase), interconversion of 5,10-methenylTHF and 10-formylTHF (methylenTHF cyclohydrolase), and reaction of formate with THF to form 10-formylTHF (10-formylTHF synthetase) are all catalyzed by a single trifunctional protein, encoded by the $MTHFD1$ gene. Conversion of methylenETHF to methylenTHF is catalyzed by methylenTHF reductase (MTHFR). The dehydrogenase and cyclohydrolase reactions are catalyzed in mitochondria by bifunctional proteins encoded by the $MTHFD2$ and $MTHFD2L$ genes, while reaction of formate with THF is catalyzed by the product of the $MTHFD1L$ gene. Because of the different redox states of the two subcellular compartments, mitochondrial folate metabolism favors generation of formate from serine and glycine, while cytoplasmic

![Fig. 1 Folate metabolism in mammalian cells. GCS = glycine cleavage system; SHMT1 and SHMT2 = serine hydroxymethyltransferase, types 1 (cytoplasmic) and type 2 (mitochondrial); MTHFR = methyleneTHF reductase; MTHFD1 = cytoplasmic trifunctional enzyme containing methyleneTHF dehydrogenase, methylenTHF cyclohydrolase and formylTHF synthetase activities; MTHFD2 and MTHFD2L = mitochondrial bifunctional proteins containing methyleneTHF dehydrogenase and methylenTHF cyclohydrolase activities; MTHFD1L = mitochondrial formylTHF synthetase; FTCD = bifunctional protein containing glutamate formiminotransferase and formiminoTHF cycloaminase activities; MTR = methionine synthase; TYMS = thymidylate synthase; DHFR = dihydrofolate reductase; GART = GAR transformylase; and AICART = AICAR transformylase. Numbers in circles indicate steps affected by inborn errors of folate metabolism]
folate metabolism favors incorporation of one-carbon units derived from mitochondrially-produced formate into 10-formylTHF and then to 5,10-methyleneTHF and 5-methylTHF, and synthesis of purines and thymidine as well as remethylation of homocysteine to form methionine.

Three types of protein capable of supporting transport across cell membranes have been identified: the reduced folate carrier (RFC), the folate receptor proteins (FRα and FRβ), and the proton-coupled folate transporter (PCFT). Folate uptake from the intestine appears to be dependent on the PCFT; although the RFC is expressed in the intestine, mutations in the gene that encodes it are not associated with deficient folate absorption. Transport of folate across the blood-brain barrier appears to require both PCFT and FRα. Cellular folate uptake appears to depend on the RFC, which supports low-affinity high-capacity uptake system, and the folate receptors, which support a low-capacity high-affinity system.

Inborn errors of folate uptake and metabolism

Hereditary folate malabsorption (OMIM 229050) Patients with this autosomal recessive disorder have a specific defect in uptake of dietary folate from the intestine. Clinical findings have included megaloblastic anemia, recurrent infections and recurrent or chronic diarrhea, and neurological abnormalities including seizures, developmental delay and mental retardation, attributed to deficiency of folate in the central nervous system (Geller et al. 2002). There is impaired folate transport in both the intestine and the blood-brain barrier at the choroid plexus. The disorder is due to mutations at the SLC46A1 gene on chromosome 17q11.2, which encodes the PCFT (Qiu et al. 2006). Fourteen different mutations at this locus have been identified, including nonsense, missense and splice site mutations. Several of these mutations have been shown to affect folate transport at low pH in an in vitro system (Qiu et al. 2006). Treatment of this disorder typically involves parenteral administration of folate, although oral administration has been successful in some cases. Reduced folates appear more effective than folic acid. It is critical to ensure that the CSF folate level is maintained above levels associated with deficiency (15 ng/ml) (Cooper 1987).

Glutamate Formiminotransferase Deficiency (OMIM 229100) Histidine catabolism results in formation of formiminoglutamate, which reacts with THF to form formiminoTHF in a reaction catalyzed by glutamate formiminotransferase; formiminoTHF is then converted to 5,10-methenylTHF by formiminoTHF cyclodeaminase (Shane and Stokstad, 1986). These two reactions (reactions 1 and 2 in Fig. 1) are catalyzed by a bifunctional protein encoded by the FTCD gene on chromosome 21q22.3. Glutamate formiminotransferase deficiency is an autosomal recessive disorder with significant clinical heterogeneity. Fewer than 20 patients have been described. Patients with this disorder are characterized by elevated serum levels of formimino glutamate, either constitutively or in response to a histidine load. Clinical findings have been variable. Several early patients were mentally retarded, but only one of the 12 subsequently described patients (Erbe 1986; Van Gennip et al. 1994; Malvagia et al. 2006). There may be two different presentations, with severely affected patients characterized by physical and mental retardation, with elevated serum folate and formiminoglutamate levels, while less severely affected individuals do not have mental retardation but have massively elevated formiminoglutamate levels. Alternatively, it has been argued that the severe phenotype in some of the early patients was the result of ascertainment bias. There have not been sufficient recent patients to determine the true phenotypic range of this disease. Mutations in the FTCD gene on chromosome 21q22.3 have been identified in patients from two families with the milder phenotype (Hilton et al. 2003).

Methylenetetrahydrofolate reductase deficiency (OMIM 236250) This is the most common of the known inborn errors of folate metabolism, with over 100 patients identified. Methylenetetrahydrofolate reductase (MTHFR; reaction 3 in Fig. 1) catalyzes the conversion of 5,10-methyleneTHF to 5-methylTHF. Patients with MTHFR deficiency are characterized by hyperhomocysteinemia and homocystinuria with low or low-normal methionine levels, reflecting decreased activity of methionine synthase, which uses 5-methylTHF as methyl group donor. Unlike patients with defects in methionine synthase itself, patients with MTHFR deficiency do not have megaloblastic anemia. Clinical manifestations of the disorder are variable, including serious disease in the first year of life leading to death, developmental delay, neurological and psychiatric disease and thrombotic events (Thomas and Rosenblatt 2005). Other patients present later in childhood, often with developmental delay and varying neurological manifestations. Asymptomatic adults have been ascertained after diagnosis of a sibling with a more severe presentation of the disorder. This autosomal recessive disorder is caused by mutations at the MTHFR gene on chromosome 1p36.3 (Goyette et al. 1994). Over 50 disease causing mutations have been identified to date. Almost all have been restricted to one or two families, although one mutation occurs at a relatively high frequency among old order Amish, apparently reflecting a founder effect (Strauss et al. 2007). A variety of treatments have been attempted in patients with MTHFR deficiency. The most effective agent
has been betaine, which is substrate for betaine homocysteine methyltransferase, which catalyzes conversion of homocysteine to methionine without requiring folate or cobalamin. The best response has been seen in patients in which therapy started either prenatally or soon after birth before neurological changes become irreversible (Strauss et al. 2007).

In addition to severe MTHFR deficiency, more subtle deficiencies in MTHFR activity have been identified. The most thoroughly studied of these is the c.677 C>T \textit{MTHFR} polymorphism, which results in about a 50-60% decrease in MTHFR specific activity in homozygous individuals (Frosst et al. 1995). This polymorphism causes change of an alanine residue in the catalytic domain to a valine, which results in a thermolabile enzyme. The frequency of the T allele varies in different populations, ranging from 11% in African Americans to 30% in Europeans and Japanese. The T allele is associated with elevation in total homocysteine levels, particularly in individuals with the TT genotype, in the presence of low serum folate levels. There is evidence that 677 T is associated with increased risk of neural tube defects when present in either the mother or the fetus. Results of studies of the association of the c.677 C>T polymorphism with other birth defects, and with a variety of other diseases including cardiovascular disease, Alzheimer disease, colon cancer, diabetes mellitus, Down syndrome, leukemia, and pregnancy complications have yielded ambiguous results. A second polymorphism, c.1298A>C, is associated with a 35% decrease in MTHFR activity. This polymorphism, which has a frequency of 30% in western Europeans and 18% in Asians, has been less well studied than the c.677 C>T polymorphism. Determination of \textit{MTHFR} status for either 677 C>T or 1298 A>C has little predictive value for any given patient and probably should not be done outside the context of clinical investigation.

**Functional methionine synthase deficiency** Activity of methionine synthase (reaction 4 in Fig. 1), which catalyzes the remethylation of homocysteine to form methionine, requires two proteins: methionine synthase itself, and the accessory protein methionine synthase reductase, which maintains the methionine synthase-bound cobalamin prosthetic group in its active fully-reduced state. Functional methionine synthase can be separated into two classes, cblE (OMIM 236270) and cblG, (OMIM 250940) which are the results of mutations that affect the function of methionine synthase reductase or methionine synthase. Both disorders are autosomal recessive.

The cblE disorder is characterized by megaloblastic anemia, cerebral atrophy, nystagmus, blindness and altered muscle tone. There is hyperhomocysteinemia and homocystinuria in the presence of low plasma methionine. Studies of patient fibroblast extracts demonstrated that methionine synthase specific activity was within the reference range when assayed under standard conditions, including exogenous reducing agent; but when the assay was performed using suboptimal levels of reducing agent activity was decreased in patient extracts but not in control extracts (Watkins and Rosenblatt 1988, 1989). This suggested a defect in a methionine synthase associated reducing system. Studies of methionine synthase from \textit{E. coli} had identified such a system, consisting of flavodoxin and flavodoxin oxidoreductase. This system does not exist in humans, but a gene encoding a protein with homology to coenzyme-binding sites of both bacterial components was identified and shown to be mutated in cblE patients. The gene, \textit{MTRR}, located on chromosome 5p15.2-15.3, encodes methionine synthase reductase, which has been shown to function in maintaining methionine synthase-bound cobalamin in its active form (Leclerc et al., 1998).

Patients with the cblG disorder have the same clinical presentation as cblE patients, although methionine synthase specific activity in fibroblast extracts is reduced under all assay conditions. The disorder is caused by mutations in the \textit{MTR} gene on chromosome 1q43, which encodes methionine synthase (Leclerc et al. 1996; Gulati et al. 1996; Watkins et al. 2002). Both disorders respond biochemically to therapy with hydroxocobalamin plus betaine with variable clinical response.

**Cerebral folate deficiency** (OMIM 613068) This disorder is characterized by decreased levels of 5-methylTHF in the central nervous system, in the presence of normal serum folate levels, indicating a specific inability to transport folate across the blood-brain barrier. There is a wide spectrum of neurological findings, including mental retardation, motor retardation and epilepsy that have been associated with cerebral folate deficiency (Ramaekers and Blau 2004; Pérez-Duenas et al. 2010; Mangold et al. 2011). Mutations in the \textit{FOLR1} gene on chromosome 11q13.4 have now been identified in a small number of families (Steinfeld et al. 2009; Cario et al. 2009; Pérez-Duenas et al. 2010). \textit{FOLR1} deficiency is inherited as an autosomal recessive trait. Treatment with folinic acid has been successful in increasing cerebral folate levels, but has had limited effectiveness in alleviating seizures.

**Dihydrofolate reductase deficiency** (OMIM 613839) Three families with deficiency of dihydrofolate reductase (reaction 5 in Fig. 1) have been described recently. Patients have presented with megaloblastic anemia, cerebral folate deficiency and a variety of neurological manifestations. Members of two families of Pakistani origin came to medical attention in the first weeks of life with seizures, developmental delay and cerebral and cerebellar atrophy.
(Banka et al. 2011). Members of the third family presented at 2–11 years; two had atypical childhood absence epilepsy (Cario et al. 2011). All patients were shown to be homozygous for mutations in the DHFR gene on chromosome 5q11.2-13.2. Treatment with folic acid has been effective in correcting biochemical abnormalities, but in some patients seizures have been resistant to this treatment.

**MethyleneTHF dehydrogenase deficiency** This disorder was identified in an infant who presented at 2 months of age with megaloblastic anemia, atypical hemolytic uremic syndrome, and severe combined immunodeficiency. There was elevation of both homocysteine and methylmalonic acid. Studies of cultured fibroblasts demonstrated decreased synthesis of methylcobalamin, the cobalamin derivative required for activity of methionine synthase. Exome sequencing resulted in identification of two mutations in the MTHFD1 gene on chromosome 14q23.3, which encodes the trifunctional enzyme that catalyzes the 5,10-methyleneTHF dehydrogenase, 5,10-methenylTHF cyclohydrolase and 10-formylTHF synthetase reactions (reactions 6, 7 and 8 in Fig. 1) (Watkins et al., 2011).

In summary, five inborn errors affecting folate transport and metabolism have been well described: hereditary folate malabsorption, glutamate formiminotransferase deficiency, methylenetetrahydrofolate reductase deficiency, methionine synthase deficiency (cblG) and methionine synthase reductase deficiency (cblIE). Recently, additional disorders have been identified: cerebral folate deficiency due to mutations in the gene encoding folate receptor α; dihydrofolate reductase deficiency; and deficiency of the trifunctional protein encoded by the MTHFD1 gene.

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**References**


