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Treatable Inborn Errors of Metabolism Due to Membrane Vitamin Transporters Deficiency

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B vitamins act as cofactors for strategic metabolic processes. The *SLC19* gene family of solute carriers has a significant structural similarity, transporting substrates with different structure and ionic charge. Three proteins of this family are expressed ubiquitously and mediate the transport of 2 important water-soluble vitamins, folate, and thiamine. *SLC19A1* transports folate and *SLC19A2* and *SLC19A3* transport thiamine. *PCFT* and *FOLR1* ensure intestinal absorption and transport of folate through the blood-brain barrier and *SLC19A25* transports thiamine into the mitochondria. Several damaging genetic defects in vitamin B transport and metabolism have been reported. The most relevant feature of thiamine and folate transport defects is that both of them are treatable disorders. In this article, we discuss the biology and transport of thiamine and folate, as well as the clinical phenotype of the genetic defects.
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Introduction

B vitamins are a class of water-soluble vitamins that play important roles in cell metabolism. Each B vitamin is either a cofactor (generally a coenzyme) for key metabolic processes or is a precursor needed to create one. As a cofactor, B vitamins participate in the metabolism of carbohydrates, amino acids, and fatty acids and have a major role in energy production. They are also involved in myelination, DNA synthesis, and neurotransmission.

B vitamins are found in whole unprocessed foods. Processed carbohydrates such as sugar and white flour tend to have lower B vitamin than their unprocessed counterparts. For this reason, the B vitamins thiamine, riboflavin, niacin, and folic acid are added back to white flour after processing in many countries.

There are several known genetic defects in vitamin B transport and metabolism causing disease in humans. For folate, riboflavin, and thiamine, genetic transport defects have been described in children. In this article, we will focus on thiamine and folate transporter defects.

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The folates and thiamine are metabolized to active forms that accumulate in cells where they sustain key metabolic reactions. They are transported into cells by a specific member of the SLC19 family.[1] SLC19A1 transports folate, and SLC19A2 and SLC19A3 transport thiamine. The proton-coupled folate transporter (PCFT; MIM*611672) is responsible for the intestinal absorption and the transport across the blood:choroid plexus (CP):cerebrospinal fluid (CSF) barrier. The folate receptor alpha (FOLR1) also mediates active transport to the brain using an endocytosis process. The mitochondrial thiamine pyrophosphate carrier (SLC25A19) enters the active form of thiamine to the mitochondria.

Two inborn errors affecting folate transport have been well studied: hereditary folate malabsorption (MIM 229050) due to mutations in PCFT[2] and cerebral folate transport deficiency (MIM 613068) due to defects in FOLR1.[3] Additionally, the following inherited defects of thiamine transport have been described: SLC19A2: thiamine-responsive megaloblastic anemia (MIM 249270),[4] SLC19A3: thiamine transporter-2 deficiency (biotin- or thiamine-responsive encephalopathy type 2) (MIM 607483),[5] SLC25A19: microcephaly Amish type (MIM 607196),[6] and SLC25A19: thiamine metabolism dysfunction syndrome 4 (progressive polyneuropathy type) (MIM 613710).[7]

Thiamine and folate transport defects across cell membranes share a common feature that is relevant from a therapeutic perspective: they are treatable disorders. In both cases, oral or intravenous supplementation or both leads to a significant and

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sustained clinical response and restores CSF and cellular concentrations in affected patients. The biological mechanism for this clinical response is unknown, although a major hypothesis is that alternative low affinity or residual transport pathways into the brain can be exploited by increasing plasma concentrations.

Folate Biology

Folate is a water-soluble B vitamin comprising several vitamers, which are compounds that act as coenzymes for cellular one-carbon metabolism.[8] Folate is essential for the synthesis of thymidine, purines, myelin, and neurotransmitters, and for the metabolism of amino acids such as homocysteine, methionine, serine, and glycine. Homocysteine remethylation to methionine leads to more than 100 methylation reactions via s-adenosylmethione.[9]

Impaired myelination of the central nervous system (CNS) is a common abnormality in inborn errors of folate transport or metabolism or both, such as hereditary folate malabsorption, FOLR1 deficiency, and severe 5,10-methylentetrahydrofolate reductase (MTHFR) deficiency. In these disorders, a relationship has been suggested between S-adenosylmethionine (SAM) deficiency and impaired myelination, the proposed mechanisms being related to a reduced methylation of lipids and proteins required for the formation and maintenance of the myelin sheaths.[100] SAM is the methyl donor in the synthesis of the key cell membrane component phosphatidylcholine from phosphatidylethanolamine. In rats, diet-induced folate deficiency depletes brain membrane phosphatidylcholine, which may be prevented by supplementation with L-methionine.[10] A reduced choline peak on spectroscopy in FOLR1 defects may be an estimation of reduced SAM and, consequently, of a decreased methylation capacity in the brain in cerebral folate transport deficiency.[11]

Early diagnosis and treatment of folate metabolism and transport defects can restore CSF 5MTHF concentrations and the methionine and S-adenosylmethionine pool within the brain, leading to myelin formation and brain growth.[3,12,13]

Folate Transport Across Cell Membranes and the CP

Folate is mainly obtained from fruits and vegetables in the form of polyglutamates that have to be transformed into monoglutamates to be transported into cells.

Two systems are responsible for the intestinal absorption of folate: the reduced folate carrier (RFC, encoded by the *SLC19A1* gene)[8] and the proton-coupled folate transporter (PCFT, encoded by the *SLC46A1* gene).[1] Both PCFT and RFC are expressed at the apical membrane of the intestinal epithelia, and the contribution of each system to total folate absorption depends on their expression and on the intestinal pH. The PCFT system acts in the proximal half of the small intestine, whereas the RFC system operates in the distal small intestine and the colon.[9] The identification of the first patients with pathogenic mutations in *SLC46A1*, which affects PCFT function, supports its key role in folate transport.[1,13]

Folate is converted to 5-methyltetrahydrofolate (5MTHF) by several enzymatic reactions. 5MTHF is the major biologically active form that functions as a cofactor in many methylation reactions. Different folate carriers and receptors participate in the cellular uptake of 5MTHF from the circulation to organs and cells.

The reduced folate carrier (RFC; SLC19A1) is an organic anion antiporter that exchanges 5MTHF with other inorganic or organic anions. It is ubiquitously expressed, and has a low affinity for folate, especially for the active-reduced forms. To date, no disease-causing mutations have been identified in the *SLC19A1* gene in patients with cerebral folate deficiency (CFD) syndrome.

Two glycosylphosphatidylinositol-anchored receptors, folate receptor alpha (FRα) and beta (FRβ), mediate endocytosis of folates after binding them with high affinity at neutral pH. FRα encoded by *FOLR1* gene is expressed in the apical border membrane of proximal renal tubular cells, in the retinal pigment epithelium, and in the CP.[2] Within the CP, PCFT is co-expressed with FOLR1 at the endosomal membrane, and it is likely that PCFT is required for FOLR1-mediated endocytosis. The fact that both genetic defects produce a failure to transport folates across the CP suggests that these transporters act in series; disruption of either of them results in the same defect.

Zhao et al[14] suggested that the PCFT might work in tandem with FRα-mediated endocytosis by exporting folate from the endosomes into the cytoplasm. Folate binding to FRα is followed by the invagination of the cell membrane containing the folate-receptor complex, the formation of an endosome, and trafficking of the vesicle in the endosomal compartment where it acidifies releasing folate from the receptor.

Recently, Grapp et al[15] elucidated the mechanism of folate transport through the CP and to the brain. They identified a unidirectional basolateral to apical transport of FRα and release of FRα from the apical membrane to the CSF. Within the CSF, FRα was found at the surface of exosomes, and FRα-exosome levels positively correlated with 5MTHF concentrations. Furthermore, FRα could be detected in the CSF of controls but was absent from patients with FOLR1 and Kearns-Sayre syndrome who had 5MTHF concentrations less than 5 nM (normal range: 40-120 nM). These findings suggest a link between CSF 5MTHF and CSF FRα and indicate a crucial role of the CP in the export and maintenance of 5MTHF and FRα in the CSF. Furthermore, these authors demonstrated that FRα-positive exosomes penetrate into brain parenchyma where they are internalized by astrocytes and neurons. These studies reveal a novel function of exosome as transport medium for folate, and that FRα-positive exosomes represent a particular attractive shuttle system for a broad variety of biomolecules and organic or inorganic compounds.

Hereditary Folate Malabsorption

Hereditary folate malabsorption is due to mutations in *PCFT*.[2] Biochemically, the disorder is characterized by profound blood