Folic acid derivatives are important biological compounds that occupy key roles in a variety of cellular metabolic pathways including the synthesis of purines and thymidine, the remethylation of homocysteine to form methionine and the synthesis and breakdown of a number of amino acids, neurotransmitters and myelin (Watkins and Rosenblatt, 2012). Folates are critical for foetal development, and folate deficiency has been associated with an increased risk of congenital and post-natal problems including neural tube, cardiovascular and limb defects; cleft lip and palate; chromosomal disorders, such as Trisomy 21 and Fragile X and some paediatric malignancies including leukaemia and neuroblastoma. Following a report by the Medical Research Council Vitamin Study Research Group (1992), maternal dietary supplementation with folic acid has been promoted in many countries, and the current UK recommendation (in accordance with WHO guidance) is for 400 µg/day of folic acid, commencing preconception and continuing up to 12 weeks gestation (NICE, 2008).

Structurally, the biologically active folates consist of a pteridine moiety linked to a variable number of glutamate molecules. Polyglutamated folates do not cross lipid membranes easily and require deconjugation to a monoglutamate before traversing the cell and mitochondrial membranes. Folate is primarily transported as 5-methyltetrahydrofolate (5-MTHF), and this is the major form of folate found in both plasma and CSF (Hyland et al., 2010). 5-MTHF reaches the CNS largely through choroid plexus epithelial cells, where transport across the cell membrane is achieved predominantly by a high-affinity, low-capacity endocytic process involving the folate receptor (FRα). This ATP-dependent mechanism results in a higher (>1.5-fold) concentration of 5-MTHF in CSF compared with plasma. The process seems to be regulated and responsive to circulating levels of 5-MTHF, with FRα receptor expression being inversely correlated to plasma concentration of 5-MTHF (Ramaekers and Blau, 2004). There are at least two other folate transport systems: a low-affinity, high-capacity reduced folate carrier and active cellular export, both of which may be exploited in the treatment of folate deficiency.

Sensitivity to an inadequate supply of folate is not limited to the developing brain, and typically, cerebral folate deficiency has its onset in early infancy, though late-onset cases have also been reported (Koenig et al., 2008; Sadighi et al., 2012). Cerebral folate deficiency (OMIM 613068) is a clinically heterogenous and aetologically diverse condition, first reported by Wevers et al. (1994) in a patient with a slowly progressive neurological disorder and subsequently defined as ‘...any neurological syndrome associated with a low cerebrospinal fluid concentration of 5-MTHF in the presence of normal peripheral folate status’ (Ramaekers and Blau, 2004). There is a specific inability to transport 5-MTHF across the blood–brain barrier, resulting in a host of neurological sequelae including seizures, delayed motor and cognitive development, autistic features, poor head growth, cerebellar ataxia, visual and hearing impairment, dyskinesia and spasticity (Pérez-Dueñas et al., 2011). An antibody-mediated process, with antibodies generated to FRα, was thought to be responsible, but more recently mutations in FOLR1, the gene encoding FRα, have been described in a number of cases of cerebral folate deficiency (Cario et al., 2009; Steinfeld et al., 2009). Other conditions associated with cerebral folate deficiency include dihydropteridine reductase deficiency; Rett’s syndrome; Aicardi-Goutiére’s syndrome; hypomyelination with atrophy of the basal ganglia; Kearns-Sayre syndrome and several other mitochondrial respiratory chain diseases (Pérez-Dueñas et al., 2011). The mechanism by which cerebral folate deficiency arises in these conditions is not entirely clear, though for mitochondrial diseases, deficient ATP production could explain the difficulty in transporting 5-MTHF by FRα receptor endocytosis.

Patients with cerebral folate deficiency often respond positively to supplementation with oral folic acid (5-formyltetrahydrofolate), in preference to folic acid. The latter is a synthesized, oxidized and biologically inactive form of folate that may competitively inhibit 5-MTHF at the FRα receptor and worsen the clinical condition. The best responses to treatment are seen when folinic acid supplementation is initiated in early childhood, though seizures, typically myoclonic astatic, may prove refractory. In some instances, parenteral, or rarely intrathecal, administration of folinic acid is necessary, particularly when intestinal malabsorption is a problem.
In this issue of *Brain*, Grapp *et al.* report their results on the molecular genetic investigation of a cohort of 72 children with a late-infantile, progressive neurological phenotype and a low concentration of 5-MTHF in CSF. Specifically, they have sequenced the *FOLR1* gene and identified mutations (including several novel mutations) in a substantial proportion of cases. As a consequence of these investigations and the detailed phenotypic history they received from participating collaborators in Germany, Finland, Italy, Switzerland, The Netherlands, the UK and the USA, they have been able to identify a particular phenotype that is likely to be associated with mutations in *FOLR1*. Grapp *et al.* found that in a group of patients (n = 14) with extremely low (<5 nmol/l) levels of CSF 5-MTHF, *FOLR1* mutations were identified in 10 (71%), and importantly, all patients with *FOLR1* mutations had extremely low CSF concentrations of 5-MTHF. All patients presented with early onset (<3 years) of symptoms of developmental delay, ataxia and seizures associated with a slow background rhythm and multifocal epileptic activity on EEG. Seizures were most often myoclonic, but atactic, tonic and flexion contractions of the trunk and neck similar to infantile spasms were also reported. MRI most often identified white matter abnormalities (delayed myelination or hypomyelination) and cerebellar atrophy. Two older patients, aged 13 and 15 years, respectively, had evidence of severe polyneuropathy.

FRα expression in model cell systems and patient fibroblasts confirmed that stop and splice site mutations resulted in no functional FRα expression, but two of the three missense mutations resulted in near-normal expression of stable FRα. Interestingly, folic acid binding in these same missense mutant cells was drastically reduced. One possible explanation was that although FRα was expressed, it was not correctly located in the cell membrane; a prerequisite for the initiation endocytosis necessary for folic acid/5-MTHF transport. Grapp *et al.* tested this mis-targeting theory by investigating the subcellular localization of wild-type and missense mutant FRα in transfected Chinese Hamster ovary cells using confocal immunofluorescence with antibodies to FRα and endoplasmic reticulum. FRα wild-type was localized to the plasma membrane, whereas mutant FRα was misdirected to a variety of intracellular compartments including the endoplasmic reticulum.

Cerebral folate deficiency is one of the few progressive neurologic disorders of childhood that is amenable to treatment. Moreover, the treatment, folicic acid, is safe, cheap and effective (to at least some degree) in most cases, with spectacular results in a few. It is, therefore, imperative to identify cerebral folate deficiency at an early stage by determination of 5-MTHF concentration in CSF. Of the multiple aetiologies of cerebral folate deficiency, mutations in the *FOLR1* gene seem to be among the most common and severe. The meticulous work by Grapp *et al.* has clarified not only the clinical features associated with *FOLR1* mutations but also the mechanism, a failure of FRα expression or membrane localization, by which folate transport into the CSF is impaired. The role that FRα misdirection plays in cerebral folate deficiency secondary to other neurologic conditions, such as mitochondrial disease, remains to be seen.

### Funding

Dr Robert McFarland’s Clinical Senior Lectureship is funded by the Department of Health (UK) and Higher Education Funding Council for England and his research is supported by the Medical Research Council (Award no: 86688).

Robert McFarland
Wellcome Trust Centre for Mitochondrial Research, Institute for Ageing and Health, Newcastle University, Newcastle upon Tyne NE2 4HH, UK

Correspondence to: Robert McFarland,
Wellcome Trust Centre for Mitochondrial Research, Institute for Ageing and Health, Newcastle University, Newcastle upon Tyne NE2 4HH, UK
E-mail: robert.mcfarland@ncl.ac.uk
Received May 28, 2012. Accepted May 28, 2012
doi:10.1093/brain/aws166

### References


