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ORIGINAL ARTICLE

Progressive ataxia and myoclonic epilepsy in a patient with a homozygous mutation in the *FOLR1* gene

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Abstract Several unrelated disorders can lead to 5-methyltetrahydrofolate (5MTHF) depletion in the cerebrospinal fluid (CSF), including primary genetic disorders in folate-related pathways or those causing defective transport across the blood-CSF barrier. We report a case of cerebral folate transport deficiency due to a novel homozygous mutation in the *FOLR1* gene, in an effort to clarify phenotype–genotype correlation in this newly identified neurometabolic disorder. A previously healthy infant developed an ataxic syndrome in the second year of life, followed by choreic movements and progressive myoclonic epilepsy. At the age of 26 months, we analyzed CSF 5MTHF by HPLC with fluorescence detection and conducted magnetic resonance (MR) imaging and spectroscopy studies. Finally, we performed mutational screening in the coding region of the *FOLR1* gene. MR showed a diffuse abnormal signal of the cerebral white matter, cerebellar atrophy and a reduced peak of choline in spectroscopy. A profound deficiency of CSF 5MTHF (2 nmol/L; NV 48-127) with reduced CSF/plasma folate ratio (0.4; NV 1.5-3.5) was highly suggestive of defective brain folate-specific transport across the blood-CSF/brain barrier. Mutation screening of *FOLR1* revealed a new homozygous missense mutation (p.Cys105Arg) that is predicted to abolish a disulfide bond, probably necessary for the correct folding of the protein. Both parents were heterozygous carriers of the same variant. Mutation screening in the *FOLR1* gene is advisable in children with profound 5MTHF deficiency and decreased CSF/serum folate ratio. Progressive ataxia and myoclonic epilepsy, together with impaired brain myelination, are clinical hallmarks of the disease.

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Introduction

Cerebral folate deficiency (CFD) syndrome is a heterogeneous neurometabolic condition characterized by low concentration of 5-methyltetrahydrofolate (5MTHF) in the cerobrospinal fluid (CSF) and by normal blood folate values (Ramaekers et al. 2005). Several unrelated processes can lead to 5MTHF depletion in the CSF. These can be divided into two main CFD syndromes: (1) a more common, milder form of deficiency identified in a broad spectrum of neurological diseases (Blau et al. 2003; Garcia-Cazorla et al. 2008; Mercimek-Mahmutoglu and Stockler-Ipsiroglu 2007; Temudo et al. 2009) and in children with serum folate receptor (FR) autoantibodies (Ramaekers et al. 2005); and (2) a severe form restricted to children with genetic conditions leading to impaired folate transport or metabolism (Skovby 2003). In the latter group, Steinfeld et al. (2009) described etiologic mutations in the candidate gene *FOLR*1 (MIM *136430) encoding the folate receptor alpha (FRα) in three children with profound CSF 5MTHF deficiency. FRα is a high-affinity transport system involved in folate transport across the blood-CSF barrier, and is the predominant mechanism for cellular incorporation at physiological 5MTHF extracellular concentrations. Recent studies by Zhao et al. (2009) suggested that the protein-coupled folate transporter (PCFT; MIM*611672) might work in tandem with FRα-mediated endocytosis by exporting folate from the endosomes into the cytoplasm, thereby playing a biological role in folate transport to the brain.

The FRα defect described by Steinfeld et al. was named cerebral folate transport deficiency, and it produced a progressive neurological disorder in the second to third year of life characterized by psychomotor regression, epilepsy and impaired brain myelination. In these patients, folinic acid supplementation ameliorated the symptoms in all cases and completely reversed the clinical picture in the less severely affected child (Steinfeld et al. 2009; Cario et al. 2009).

Here, we report a new patient with cerebral folate transport deficiency caused by a novel homozygous missense mutation in the *FOLR1* gene. We describe clinical, neuroimaging and genetic features that contribute to the clarification of genotype-phenotype correlations of this rare neurometabolic disease.

Patient

This 7-year-old boy was the second child of healthy consanguineous parents (first cousins) from Gambia. He was born at term after a normal pregnancy and delivery. Anthropometric data at birth were as follows: weight 3,380 g (50th percentile), height 49.5 cm (50th percentile) and head circumference 34 cm (25th percentile). He showed normal development in the first year of life, together with normal growth and head circumference. Progressive ataxia developed in the second year of life, manifesting with a broad-based gait, instability and jerky tremor in upper limbs. Examination also revealed cognitive impairment and language delay. He suffered a febrile tonic-clonic status at the age of 21 months and was treated with valproic acid. Neurological deterioration progressed, and he was admitted to the hospital at the age of 26 months for further evaluation. During hospitalization, the most striking features were continuous and generalized choreic movements and multiple, asynchronous myoclonic jerks. Involuntary movements prevented standing and ambulation. He had no social smile, lacked language communication, and only produced incomprehensible sounds. He suffered daily tonic and myoclonic attacks. Prolonged tonic seizures were associated with apnoea and desaturation. The electroencephalogram (EEG) registered slow high amplitude background activity, frequent myoclonic jerks and tonic seizures (Fig. 1). Clobazam was added to valproic acid with partial improvement in myoclonus and seizure activity. Significantly prolonged latencies were recorded during visual evoked potentials. Other neurophysiologic studies (electroretinography, brainstem auditory evoked potentials, neurography and electromyography) disclosed normal findings. At the same time, blood and CSF samples were collected for biochemical and genetic analysis and neuroimaging study was performed.

Methods

Magnetic resonance examination

MRI examinations were performed on a 1.5-T magnet system (Signa Excite HD, Milwaukee, WI, USA). We obtained sagittal SE T1-weighted, axial SPGR 3D T1-weighted, coronal fast-spin echo with fluid-attenuated inversion recovery (FLAIR) and axial FSE T2-weighted imaging. Single-voxel hydrogen MR spectroscopy (PROBE, GE, Milwaukee, WI, USA) was performed after MRI using the point resolved spectroscopy (PRESS) technology with repetition time TR 1,500 ms and echo time TE 135 ms, focused on the right posterior parietal white matter.

Biochemical analysis

Plasma and CSF samples were collected on the same day following a protocol that we established to ensure assay reliability (Ormazabal et al. 2005). Following lumbar puncture, the CSF was immediately stored at −80°C until

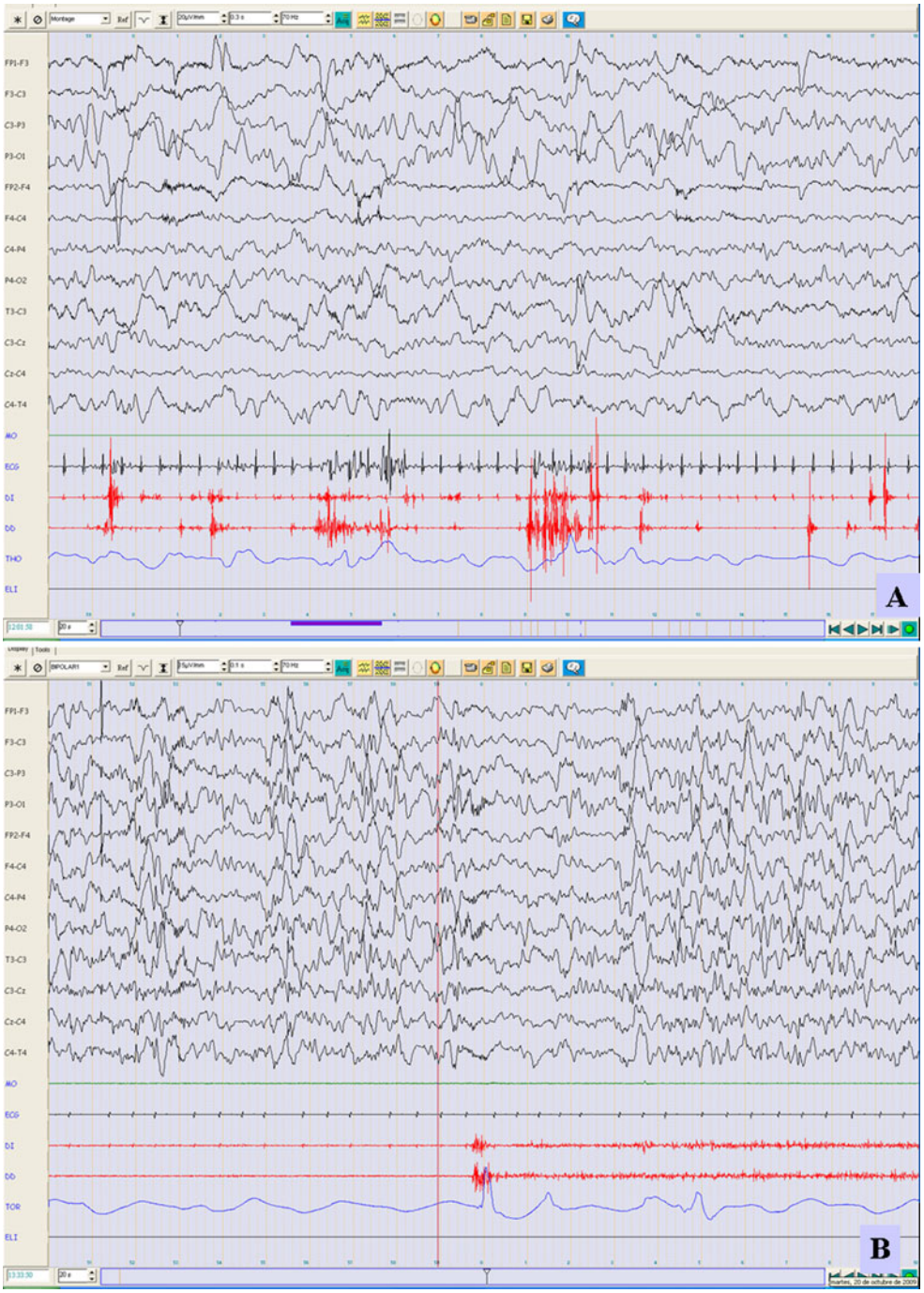

Fig. 1 **a** Multifocal myoclonic jerks on deltoides-electromyographic recording which was not associated with an electroecephalographic activity. **b** Fast generalized discharge predominating in the parieto-occipital lobes, with a short and simultaneous tonic muscle contraction on deltoids-electromyography. Note changes in heart rate and respiratory frequency during the discharge

analysis. 5MTHF, pterins (neopterin, biopterin) and biogenic amine metabolites (5-hydroxyindoleacetic acid (5HIAA), and homovanillic acid (HVA)) were analyzed by high performance liquid chromatography (HPLC) with electrochemical and fluorescence detection as previously reported by our laboratory (Ormazabal et al. 2005, 2006). Total serum folate was analyzed by automated chemiluminescent immunoassays (ADVIA Centaur; Bayer, Tarrytown, NY, USA). Plasma total homocysteine was analyzed by HPLC with fluorescence detection (Vilaseca et al. 1997).

Mutation screening of the *FOLR1* gene

Genomic DNA from the patient and his father was prepared from peripheral blood leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Genomic DNA from the mother was obtained from saliva using the Oragene DNA Self-Collection Kit (DNA Genotek, Canada). The entire coding sequence, splicing junctions and branch sites of the *FOLR1* gene were PCR-amplified and sequenced using standard methods. For PCR conditions and primers see

Supplementary Table 1. The cDNA sequence numbering follows RefSeq Accession No. NM_016725.2 (transcript variant 1), with nucleotide 211, the adenine of the ATG start codon, corresponding to position +1. The protein numbering is based on the reference sequence NP 057937.1. Mutation nomenclature follows HGVS guidelines (www.hgvs.org).

Samples from the patient and the parents were obtained in accordance with the Helsinki Declaration of 1964, revised in 2000. Informed consent was obtained from parents, and the Ethics Committee of the Hospital Sant Joan de Déu approved the study.

Results

MRI and MRS

MRI showed a diffuse abnormal signal of the white matter in cerebral hemispheres. The posterior limb of the internal capsule, corticospinal tracts, corpus callosum and cerebellar white matter were preserved (Figs. 2 and 3). The study also showed focal areas of hypointensity on T1 and hyperintensity on T2 at the periventricular white matter and centrum semiovale (Figs. 3 and 4). There was an enlargement of the cisterna magna and the cerebellar folia with mild hypoplasia of the vermis. Spectroscopy in right parietal subcortical white matter detected a reduced ratio for choline when compared with an age-matched control (Fig. 5).

Biochemical data

The child was selected from a population of 71 patients with reduced 5MTHF concentrations in CSF followed up at the Paediatric Neurology Department of Sant Joan de Déu Hospital (Barcelona, Spain). Selection was based on the presence of a profound deficiency of CSF 5MTHF (a decrease greater than 60% below the lower limit of our reference values) and reduced CSF/plasma folate ratio, which suggested a defective brain specific-folate transport across the blood-CSF/brain barrier (Table 1). The concentrations of biogenic amine metabolites and pterins in CSF were normal. In blood, a moderate deficiency of total serum folate and mild hyperhomocysteinemia were detected. Vitamins and whole blood count results were normal (Table 1).

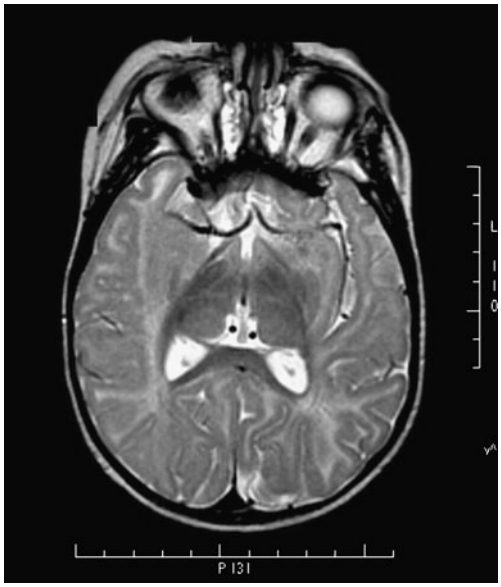

Fig. 2 FSE T2 (3500/84) Axial acquisition showed difuse high signal intensity of the subcortical and periventricular white matter. The splenium of the corpus callosum and corticospinal tracts were preserved

Table 1 Biochemical values in blood and CSF of the patient compared with age-matched reference values

	Patient	Reference values (range)
CSF analysis		
5MTHF	2	48–127 nmol/L
HVA	540	344–906 nmol/L
5HIAA	212	170–490 nmol/L
HVA/5HIAA ratio	2.5	1.1–3.5
Neopterin	12	8–43 nmol/L
Biopterin	21	8–54 nmol/L
Blood analysis		
Hemoglobin	10.8	10.5–13.5 g/dL
Erythrocytes	3.9	3.7–5.3 million/mm^3
Mean Corpuscular Volume	85	72–86 fl
Total serum folate	5.1	≥13 nmol/L
CSF 5MTHF/serum folate ratio	0.4	1.5–3.5
B6	13.4	3.6–18 μg/L
B12	665	198–996 pmol/L
tHcy	11.5	3.3–8.3 μmol/L

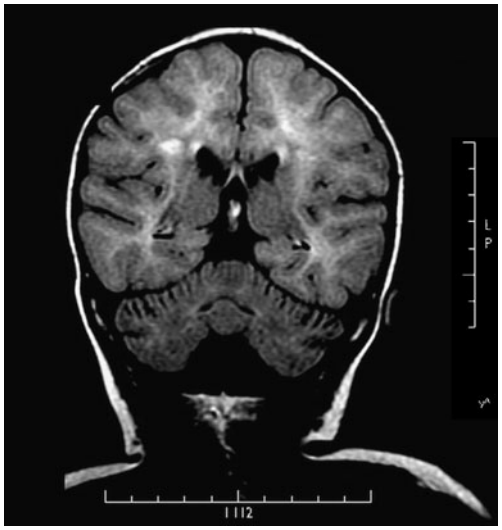

Fig. 3 FLAIR (8202/189/2000) Coronal image. Diffuse hyperintensity of the white matter. A focal area of even higher signal was identified close to the right ventricle. Enlarged folia of the cerebellar hemispheres and mild atrophy of the vermis were also observed

Genetics

Mutation screening of the *FOLR1* gene, encoding the folate receptor α (FRα), in the patient revealed a novel homozygous missense mutation in the second coding exon of the gene (c.313T>C) that causes a substitution of cysteine to arginine (p.Cys105Arg).

Both parents were heterozygous carriers of the same variant (Fig. 6). The change was not found in a screening of 105 healthy individuals. A prediction of all disulfide bounds in FRα was conducted using the online tool DCON

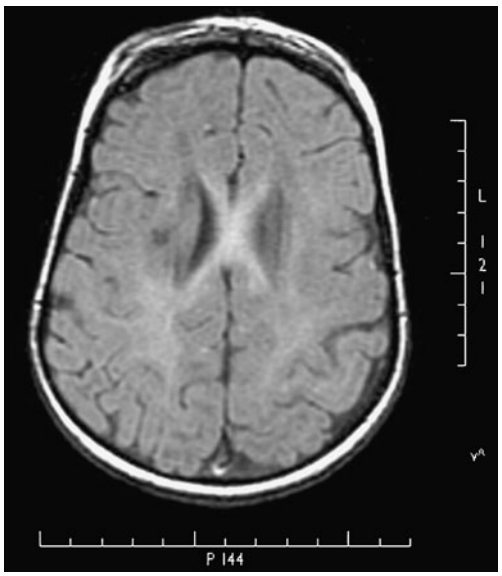

Fig. 4 SPGR T1 3D IR (36/15) Axial image at the level of the centrum semiovale. The focal area of higher intensity in the FLAIR image showed a shorter T1 signal than the surrounding white matter

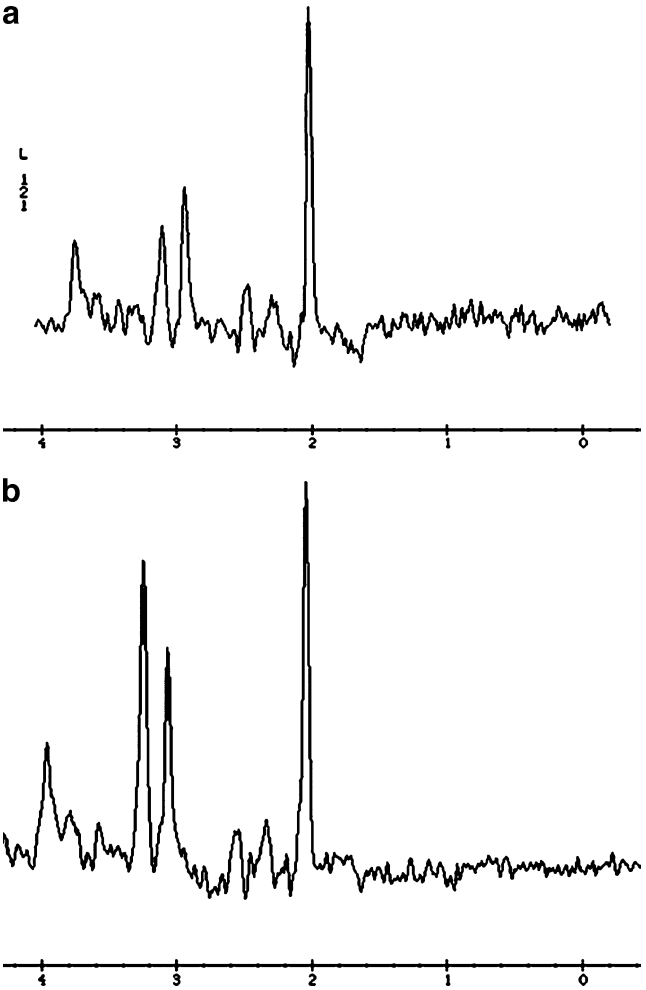

Fig. 5 AX-PROBE-SV PRESS (TR 1500 / TE 135). **a** Our patient. **b** Age-matched control. In vivo magnetic resonance spectroscopy showed a Cho/tCr ratio of 0.74 in our patient versus 1.52 in the control patient. No significant reduction was observed in the Ins/tCr or NAA/tCr ratios

(gpcr.biocomp.unibo.it) (Fariselli and Casadio 2001). The scoring results showed that the predicted disulfite bond p. Cys105-Cys109 is one of the most reliable in the protein, suggesting that the cystein residue at position 105 is likely to have a role in correct protein folding. Furthermore, alignments of the FRα protein across 38 species show complete conservation of this cysteine residue (Fig. 6).

Therapeutic trial with folinic acid supplementation

A trial with folinic acid (30 mg/day) at the age of 26 months completely controlled seizures in 1 month and significantly reduced myoclonic jerks and choreic movements. Unfortunately, the child returned to Gambia and no further clinical follow-up was recorded. We could contact the family again at the age of 7 years, but they had stopped folinic administration and the child suffered from recurrent seizures triggered by febrile episodes, lacked any language

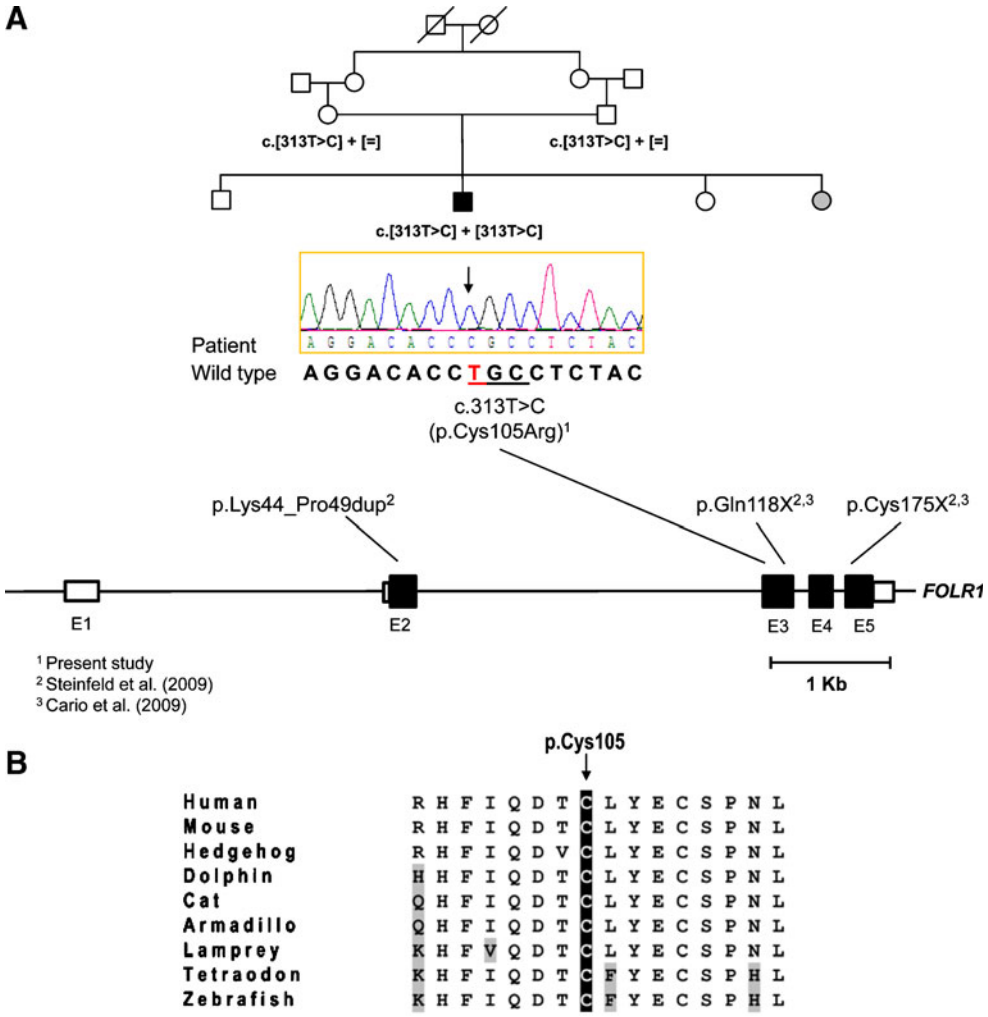

Fig. 6 **a** Sequence analysis of a PCR product containing exon 3 of the *FOLR1* gene in a patient with cerebral folate transport deficiency (*filled symbol*) and in his non-affected consanguineous parents. The patient is homozygous for the c.313T>C (p.Cys105Arg) mutation and both parents are heterozygous carriers of this variant. Another sibling showing a similar disease phenotype is still pending an accurate diagnosis (*gray symbol*). *Below*, schematic representation of the *FOLR1* gene, encoding the folate receptor α (FRα), with the three mutations previously found by other authors in patients with cerebral folate deficiency. Exons are indicated as *boxes*, with the coding sequences in *black*. **b** Protein alignment performed with ClustalW (align.genome.jp). The p.Cys105 residue of the FOLR1 protein is conserved across evolution in the 38 species analysed (only 9 sequences are shown). Non-conserved amino acids are indicated in *gray*

skills and had no ambulation. We re-introduced oral folinic acid supplementation (4 mg/kg/day) and 8 months later the parents reported that the patient, who is still living in Gambia, is seizure-free and has achieved a modest improvement in his psychomotor development.

Discussion

This report describes clinical, biochemical and genetic features of a patient with a novel homozygous mutation in the *FOLR1* gene and therefore contributes to clarify the phenotype of cerebral folate transport deficiency. Clinically, a normal period of development preceded the insidious onset of symptoms in the second year of life. He suffered a progressive complex motor disorder beginning with cerebellar ataxia and followed by generalized chorea and continuous myoclonic jerks. Tonic and myoclonic seizures, together with cognitive deterioration, completed the devastating clinical picture. The disease could be included in the heterogeneous group of genetic encephalopathies that may present with progressive myoclonic epilepsy in early childhood (Aicardi et al. 2004). The clinical data reported so far are scarce but basically in accordance with our patient's phenotype. Authors have described severely motor- and mentally-handicapped children with drug-refractory epilepsy after a period of normal neurodevelopment in infancy (Steinfeld et al. 2009). Tremor and ataxia were reported in two German siblings. Myoclonic-astatic seizures were present in the most affected sibling (Cario et al. 2009).

MRI examination in our patient disclosed a disturbance of the process of myelination diffusively affecting white matter of the cerebral hemispheres. In contrast, there was a preservation of myelin that had been deposited early in life (in the cerebellum, corticospinal tracts, posterior limb of the internal capsule, splenium of corpus callosum and striatum), endowed with greater metabolic stability (Van der Knaap and Pouwels 2005). The signal abnormalities detected in the white matter of our patient suggested

combined mechanisms of impaired deposition of myelin and myelin loss. The reduced peak of choline detected in spectroscopy indicated decreased myelin membrane synthesis and turnover (Van der Knaap and Pouwels 2005). White matter abnormalities were similar to those previously reported in children with cerebral folate transport deficiency (Steinfeld et al. 2009). In our patient, MRI also detected signs of moderate cerebellar atrophy, a finding not described in the other patients.

Impaired myelination of the CNS is a common abnormality in other inborn errors of folate transport and/or metabolism, such as hereditary folate malabsorption 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 (Surtees 1998). 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 (Troen et al. 2008). Hence, reduced choline peak on spectroscopy may be an estimation of reduced SAM and, consequently, of a decreased methylation capacity in the brain in cerebral folate transport deficiency.

The presence of severe 5MTHF deficiency in CSF and decreased CSF/plasma folate ratio in our patient suggested a defective brain specific-folate transport across the blood-CSF/brain barrier. However, it is important to note that these biochemical abnormalities are not specific of a genetic FRα defect, as they can be present in severe MTHFR deficiency (Skovby 2003), Kearns-Sayre sydrome (KSS) (Serrano et al. 2010) and other cerebral degenerations (personal observations). Hence, delineating the clinical phenotype of cerebral folate transport deficiency is essential for an accurate selection of candidates to be screened for *FOLR1* mutations.

All the children reported with cerebral folate transport deficiency and *FOLR1* mutations had a decrease in CSF 5MTHF concentration greater than 80% below the lower limit of reference values. This is in contrast with the most common partial 5MTHF deficiency associated with several unrelated encephalopathies of childhood, which may represent up to 90% of CFD syndrome (unpublished results).

In CSF, no biogenic amines or pterin defects were detected in our patient or in the patients previously reported with FRα defects. The fact that patients with profound 5MTHF deficiency from different aetiologies, such as KSS or severe MTHFR deficiency, may show increased, normal or even low biogenic amine concentrations suggests that the relationship between these metabolites may be more complex than previously reported (Surtees et al. 1994; Serrano et al. 2010).

Concerning biochemical analysis in blood, our patient showed a mild reduction of total serum folate concentrations and mild hyperhomocysteinemia. These findings normalized after 1 month of folinic acid therapy. FRα is distributed at epithelial cells, such as choroid plexus, lung, thyroid, and renal tubular cells, and therefore may have a role in folate transport to other organs and tissues. This could explain the systemic abnormalities on folate metabolism detected in our patient, as it happens in the *Folr1* knock-out mouse model (Taparia et al. 2007). Furthermore, owing to the interference of valproate in folate metabolism and transport (Opladen et al. 2010), a negative effect of this treatment in our patient's phenotype is also possible.

CFD syndrome encompasses a heterogeneous group of disorders which may be amenable to therapeutic supplementation with folinic acid or its precursors. In patients with cerebral folate transport defect due to *FOLR1* mutations, and in KSS, high doses of folinic acid (5 mg/kg/day) may significantly improve cerebral white matter abnormalities and neurological symptoms (Pineda et al. 2006; Cario et al. 2009). The variable therapeutic efficacy reported so far in patients with CFD syndrome may reflect the clinical heterogeneity underlying 5MTHF deficiency, which may be due to disorders with no primary relation to folate transport or metabolism (Temudo et al. 2009; Moretti et al. 2008).

This is the third report of *FOLR1* mutations underlying cerebral folate transport deficiency. We have identified a novel homozygous missense mutation (p.Cys105Arg) in a patient with consanguineous parents from Gambia. Several facts suggest that the identified change is indeed a disease-causing mutation. First, the mutation was not found in a screening of more than 200 control chromosomes from healthy individuals nor in the main public SNP databases (NCBI Entrez SNP: www.ncbi.nlm.nih.gov/snp; HapMap: ww.hapmap.org; ENSEMBL: www.ensembl.org) indicating that it may not be a neutral polymorphism present in the general population. Second, the amino acid residue p.Cys105 is highly conserved across evolution, suggesting functional/structural relevance. Third, the change from a neutral amino acid (cysteine) to a positively charged residue (arginine) is predicted to affect the formation of a disulfide bond and is likely to abolish the correct protein folding. And finally, the mutation was found at homozygosity in a consanguineous family in a gene that had previously been related to the disorder. However, further functional characterization of the mutant allele is needed to confirm these assumptions.

In conclusion, based on our personal experience and on that of previously reported patients, we propose that cerebral folate transport deficiency should be ruled-out in patients presenting with the following clinical and biological signs: (1) a homogeneous phenotype characterized by psychomotor regression, ataxia, epilepsy and brain hypomielination); (2) a profound CSF 5MTHF deficiency with decreased CSF/plasma folate ratio; and (3) clinical response to high doses (up to 5 mg/kg/day) of folinic acid. These criteria may be useful to distinguish this genetic condition from secondary or symptomatic forms of CFD syndrome. Because this is a potentially treatable disorder and genetic testing can be provided to the family, screening for mutations in the *FOLR1* gene is advisable in patients fulfilling the above-mentioned signs.

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References

Aicardi J, Arzimanoglou A, Guerrini R (2004) Myoclonic epilepsies associated with progressive degenerative disorders: progressive myoclonic epilepsies. In: Aicardi J, Arzimanoglou A, Guerrini R (eds) Aicardi's epilepsy in children. Lippincott Williams & Wilkins, Philadelphia, pp 81–87

Blau N, Bonafé L, Krägeloh-Mann I et al (2003) Cerebrospinal fluid pterins and folates in Aicardi-Goutières syndrome: a new phenotype. Neurology 61:642–647

Cario H, Bode H, Debatin KM, Opladen T, Schwarz K (2009) Congenital null mutations of the FOLR1 gene: a progressive neurologic disease and its treatment. Neurology 73:2127–2129

Fariselli P, Casadio R (2001) Prediction of disulfide connectivity in proteins. Bioinformatics 17:957–964

Garcia-Cazorla A, Quadros EV, Nascimento A et al (2008) Mitochondrial diseases associated with cerebral folate deficiency. Neurology 70:1360–1362

Mercimek-Mahmutoglu S, Stockler-Ipsiroglu S (2007) Cerebral folate deficiency and folinic acid treatment in hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC) syndrome. Tohoku J Exp Med 211:95–96

Moretti P, Peters SU, Del Gaudio D et al (2008) Brief report: autistic symptoms, developmental regression, mental retardation, epilepsy, and dyskinesias in CNS folate deficiency. J Autism Dev Disord 38:1170–1177

Opladen T, Blau N, Ramaekers VT (2010) Effect of antiepileptic drugs and reactive oxygen species on folate receptor 1 (FOLR1)-dependent 5-methyltetrahydrofolate transport. Mol Genet Metab Jun 16. In press

Ormazabal A, García-Cazorla A, Fernández Y, Fernández-Alvarez E, Campistol J, Artuch R (2005) HPLC with electrochemical and fluorescence detection procedures for the diagnosis of inborn errors of biogenic amines and pterins. J Neurosci Methods 142:153–158

Ormazabal A, García-Cazorla A, Pérez-Dueñas B et al (2006) Determination of 5-methyltetrahydrofolate in cerebrospinal fluid of paediatric patients: reference values for a paediatric population. Clin Chim Acta 371:159–162

Pineda M, Ormazabal A, López-Gallardo E et al (2006) Cerebral folate deficiency and leukoencephalopathy caused by a mitochondrial DNA deletion. Ann Neurol 59:394–398

Ramaekers VT, Rothenberg SP, Sequeira JM et al (2005) Autoantibodies to folate receptors in the cerebral folate deficiency syndrome. N Engl J Med 352:1985–1991

Serrano M, García-Silva MT, Martin-Hernandez E et al (2010) Kearns-Sayre syndrome: cerebral folate deficiency, MRI findings and new cerebrospinal fluid biochemical features. Mitochondrion Apr 11. [Epub ahead of print]

Skovby F (2003) Disorders of sulfur amino acids. In: Blau N, Duran M, Blaskovics ME, Gibson KM (eds) Physician's guide to the laboratory diagnosis of metabolic diseases, 2nd edn. Springer, Berlin, pp 243–260

Steinfeld R, Grapp M, Kraetzner R et al (2009) Folate receptor alpha defect causes cerebral folate transport deficiency: a treatable neurodegenerative disorder associated with disturbed myelin metabolism. Am J Hum Genet 85:354–363

Surtees R (1998) Demyelination and inborn errors of the single carbon transfer pathway. Eur J Pediatr 157:S118–S121

Surtees R, Heales S, Bowron A (1994) Association of cerebrospinal fluid deficiency of 5-methyltetrahydrofolate, but not S-adenosylmethionine, with reduced concentrations of the acid metabolites of 5-hydroxytryptamine and dopamine. Clin Sci (Lond) 86:697–702

Taparia S, Gelineau-van Waes J, Rosenquist TH, Finnell RH (2007) Importance of folate-homocysteine homeostasis during early embryonic development. Clin Chem Lab Med 45:1717–1727

Temudo T, Rios M, Prior C et al (2009) Evaluation of CSF neurotransmitters and folate in 25 patients with Rett disorder and effects of treatment. Brain Dev 31:46–51

Troen AM, Chao WH, Crivello NA et al (2008) Cognitive impairment in folate-deficient rats corresponds to depleted brain phosphatidylcholine and is prevented by dietary methionine without lowering plasma homocysteine. J Nutr 138:2502–2509

Van der Knaap MS, Pouwels PJW (2005) Magnetic resonance spectroscopy: basic principles and application in white matter disorders. In: van der Knaap MS, Valk J (eds) Magnetic resonance of myelination and myelin disorders, 3rd edn. Springer, Berlin, pp 859–880

Vilaseca MA, Moyano D, Ferrer I, Artuch R (1997) Total homocysteine in pediatric patients. Clin Chem 43:690–692

Zhao R, Min SH, Wang Y, Campanella E, Low PS, Goldman ID (2009) A role for the proton-coupled folate transporter (PCFT-SLC46A1) in folate receptor-mediated endocytosis. J Biol Chem 284:4267–4274