Genetic assessment and folate receptor autoantibodies in infantile-onset cerebral folate deficiency (CFD) syndrome


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ABSTRACT

Introduction: Cerebral folate deficiency (CFD) syndromes are defined as neuro-psychiatric conditions with low CSF folate and attributed to different causes such as autoantibodies against the folate receptor-alpha (FR) protein that can block folate transport across the choroid plexus, FOLR1 gene mutations or mitochondrial disorders. High-dose folic acid treatment restores many neurologic deficits.

Study aims and methods: Among 36 patients from 33 families the infantile-onset CFD syndrome was diagnosed based on typical clinical features and low CSF folate. All parents were healthy. Three families had 2 affected siblings, while parents from 4 families were first cousins. We analysed serum FR autoantibodies and the FOLR1 and FOLR2 genes. Among three consanguineous families homozygosity mapping attempted to identify a monogenetic cause. Whole exome sequencing (WES) was performed in the fourth consanguineous family, where two siblings also suffered from polyneuropathy as an atypical finding.

Results: Boys (72%) outnumbered girls (28%). Most patients (89%) had serum FR autoantibodies fluctuating over 5–6 weeks. Two children had a genetic FOLR1 variant without pathological significance. Homozygosity mapping failed to detect a single autosomal recessive gene. WES revealed an autosomal recessive polynucleotide kinase 3′phosphatase (PNKP) gene abnormality in the siblings with polyneuropathy.

Discussion: Infantile-onset CFD was characterized by serum FR autoantibodies as its predominant pathology whereas pathogenic FOLR1 gene mutations were absent. Homozygosity mapping excluded autosomal recessive inheritance of any single responsible gene. WES in one consanguineous family identified a PNKP gene abnormality that explained the polyneuropathy and also its contribution to the infantile CFD syndrome because the PNKP gene plays a dual role in both neurodevelopment and immune-regulatory function. Further research for candidate genes predisposing to FRα-autoimmunity is suggested to include X-chromosomal and non-coding DNA regions.

1. Introduction

Folates are essential cofactors for a multitude of biological processes such as homocysteine metabolism, one-carbon group transfer reactions, synthesis of neurotransmitters and of purines and thymidine [1–4].

The folate receptor (FOLR) genes are part of a gene family located on chromosome region 11q13.3–q13.5 including a so-called adult gene (FOLR1; folate receptor alpha), a foetal gene (FOLR2; folate receptor beta) and pseudo-genes. FOLR1 and FOLR2 are functional and are characterized by alternative splicing and numerous tissue specific transcripts that show variation in the 5′UTR region [4–6]. The folate receptor (FR) proteins are membrane proteins that are attached by a GPI anchor to the epithelial cell membrane at the basal side of choroid plexus and function to internalize folate by an endocytotic process for its transfer to the spinal fluid and neural tissues [8]. The transport of folate across the choroid plexus epithelial cells is reported to be mediated by exosomes containing the folate receptor, that are secreted...
at the apical side of these cells into spinal fluid where these exosomes traverse the ependymal linings and are subsequently delivered to neural cells in the brain [9,10]. Similar mechanisms mediate the transfer of folates across the placental-foetal barriers [11]. During foetal development, FOLR2 is also expressed by the choroid plexus cells while from the age of 4–6 months onwards a switch to FOLR1 expression is suspected, which then serves as the main membrane-attached protein for folate transport to the CNS [12]. FOLR1 has been studied in patients with hyper homocysteinemia and numerous variants have been identified [12].

Since 2009, an autosomal recessive inherited CFD syndrome caused by nonsense, missense and splice mutations of the FOLR1 gene has been identified in eleven patients. The onset of first signs and symptoms, have been reported to develop after the first year of life with progressive movement disturbance, psychomotor decline, and epilepsy. All eleven patients showed severe depletion of folate concentration in their cerebrospinal fluid (CSF) and brain magnetic resonance imaging (MRI) demonstrated profound hypomyelination [13,14].

Prior to these reports, we had defined and identified a clinically recognizable syndrome with infantile-onset called the infantile-onset Cerebral Folate Deficiency (CFD) syndrome [15–17]. Clinical features manifest from the age of 4–6 months and develop over the next two years to the full clinical phenotype. First symptoms and signs appear around 4 to 6 months with agitation, unrest and insomnia followed by deceleration of head growth, psychomotor retardation with hypotonia and ataxia, distal pyramidal signs and in one third of patients, development of dyskinesias and/or epileptic seizures. If left untreated, bilateral hearing and visual loss develops at a later stage. In a proportion of these patients low-functioning autism was found as an additional feature.

Early detection of first clinical manifestations is critical since this may help to diagnose and treat these patients at an early age, which improves outcome. In the majority of patients with confirmation of low CSF folate levels, we detected the presence of specific serum autoantibodies directed against the FOLR1 encoded FR antigen that can block folate binding with subsequent impaired folate transfer across the choroid plexus. Several families had two or more affected siblings with infantile-onset CFD syndrome, strongly suggesting a familial genetic component in the pathogenesis of this disorder.

Therefore, in this study on 36 newly diagnosed infantile-onset CFD patients from 33 families manifesting typical clinical features, we performed a systematic analysis of the two folate receptor genes, FOLR1 and FOLR2. Both genes (adult and foetal form) were studied by direct sequencing. Analysis was restricted to the coding regions and intron-exon boundaries [18].

In three consanguineous families where the parents were first-line cousins and had one child suffering from the infantile-onset CFD syndrome, we performed homozygosity mapping to detect autosomal recessive inherited monogenetic candidate genes.

In one other consanguineous family with the infantile-onset CFD syndrome, unusual additional findings consisted of a severe progressive polyneuropathy present in two siblings (parents first-cousins). Whole exome sequencing (WES) was performed in this family to identify possible genetic factors responsible for this unusual polyneuropathy but also acting as important determinants in the pathogenesis of the infantile-onset CFD phenotype.

For each patient we analysed simultaneously serum samples on several occasions for the presence of FR autoantibodies of the blocking type. In eligible patients serum FR antibodies have been determined at one-week intervals over a period of 5 to 6 weeks.

2. Patients and methods

2.1. Patient characteristics

Thirty-three previously unreported families with the infantile-onset CFD syndrome were identified. There were 10 girls and 26 boys. The age at diagnosis varied between 1 and 24 years (mean ± SD: 6.16 ± 5.25).

Two affected siblings were found in 2 non-consanguineous families and in 1 family where parents were first cousins. In three other families with 1 affected child, parents were first cousins. According to previously reported criteria, we defined the clinical picture of infantile-onset CFD syndrome to be characterized by the presence of at least 3 out of 7 clinical key features in the presence of low spinal fluid folate and normal folate levels outside the central nervous system (Fig. 1).

In this study, the clinical features started from the age of 4 to 6 months with irritability, unrest and insomnia (55%), deceleration of head growth from the age of 6 months (58%), neurodevelopmental delay (100%) with hypotonia and ataxia (100%), pyramidal deficits in the lower limbs (41%), dyskinesia's (33%) and epileptic seizures (58%). Finally, 14 out of 36 patients with CFD manifested clearly autistic signs and symptoms (39%). Among these patients, four girls manifested features of Rett syndrome in the absence of genetic abnormalities of the MECP2 gene or other genes associated with the Rett phenotype [18].

For each individual patient, diagnosis of infantile-onset CFD syndrome was confirmed after finding diminished CSF N5-methyltetrahydrofolate values (mean ± SD: 24.7 ± 15.2 nM and range: 0–52, compared to 99 healthy controls: 82 ± 31.3 nM and range for healthy children below 5 years between 63 and 182 nM) in the presence of normal values for complete blood count, serum and red blood cell folate, vitamin B12 and homocysteine.

For each child, conditions resembling infantile CFD syndrome could be ruled out using brain imaging, metabolic work-up, serum sialotransferrin testing and MECP2 gene analysis. Further details of the differential diagnosis are outlined in Table 2.

2.2. FOLR1 and FOLR2 gene analysis

After approval of this study by the Institutional Review Board and informed consent, patients were selected by the pediatric neurologists using the inclusion criteria defined above. For each patient, blood was drawn and genomic DNA was isolated from lymphocytes by the classical phenol/chloroform procedure. The coding region of the FOLR1 and FOLR2 genes were analysed by direct sequencing. Regions of interest were amplified by PCR. The FOLR1 coding sequence was defined based on accession number NM_016725.2 GRCh37 (Hg19). Primers for amplification were designed with Primer3 software (see Table 1). For the FOLR2 gene (NM_000803.4) we used primers published previously [18].

PCR was performed using 4 primer pairs for FOLR1 gene and 3 Primer pairs for FOLR2 gene. PCR was performed in 25 μl volumes. The amplification mixture included 1x PCR buffer (Roche), 1.5 mM MgCl2 Solution (Roche), 0.8 mM of dNTP (Roche), 0.6 μM of forward primer,
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