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Undetectable Levels of CSF Amyloid-β Peptide in a Patient with 17β-Hydroxysteroid Dehydrogenase Deficiency

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Abstract. 17β-hydroxysteroid dehydrogenase 10 (HSD10) deficiency is a rare X-linked inborn error of isoleucine catabolism. Although this protein has been genetically implicated in Alzheimer's disease pathogenesis, studies of amyloid-β peptide (Aβ) in patients with HSD10 deficiency have not been previously reported. We found, in a severely affected child with HSD10 deficiency, undetectable levels of Aβ in the cerebrospinal fluid, together with low expression of brain-derived neurotrophic factor, α-synuclein, and serotonin metabolites. Confirmation of these findings in other patients would help elucidating mechanisms of synaptic dysfunction in this disease, and highlight the role of Aβ in both early and late periods of life.

Keywords: Amyloid-β peptide, cerebrospinal fluid, childhood, HSD10 deficiency, inborn errors of metabolism, neurotransmitters, synaptic proteins

INTRODUCTION

17β-hydroxysteroid dehydrogenase (HSD10) is a multifunctional mitochondrial enzyme with complex roles [1, 2]. HSD10 deficiency is an X-linked disease (MIM300256) caused by mutations in *HSD17B10* gene [3]. This disease is characterized by a clinical picture very different from other organic acidurias: patients do not develop metabolic crises, but they follow a neurodegenerative course associated with mitochondrial dysfunction [4], progressive loss of skills, epilepsy, optic atrophy, retinopathy, deafness, and movement disorders [2].

HSD10 is a protein implicated in the pathogenesis of Alzheimer's disease (AD) [5, 6]. Although HSD10 has an affinity for amyloid-β peptide (Aβ), no studies

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regarding *in vivo* quantification of Aβ have been reported in patients with HSD10 deficiency. Moreover there are no studies concerning normal cerebrospinal fluid (CSF) Aβ values in the pediatric age. We aimed to analyze the expression of Aβ in the CSF of an affected child, compared to a control pediatric population. In order to gather more information about synaptic mechanisms in this disease, neurotransmitters, brain-derived neurotrophic factor (BDNF; associated with dendritic growth, and serotoninergic transmission) and α-synuclein (AS; associated with neurotransmitter release, dopaminergic modulation and neurodegeneration) were also included in our study.

PATIENT AND METHODS

Clinical report

The patient is the first child of healthy non-consanguineous parents. Pregnancy, peripartum, and first year of life were uneventful (normal psychomotor development: he could walk with support and was able to say some words). At 13 months, and within the context of fever and diarrhea, he developed psychomotor regression (inability to sit unsupported, loss of normal use of hands), and disclosed abnormal fast erratic ocular movements, non-epileptic myoclonus, and irritability. Plasma ammonia and amino acids were normal but lactate concentration was up to 3 mmol/l (NV: 0.66–1.88 mM/l). Brain MRI was normal. Because of the initial symptoms, opsoclonus-myoclonus syndrome was suspected and dexamethasone (0.8 mg/kg/d IV for 6 days) was started. Although the patient showed slight improvement, ocular fundus revealed optic atrophy and a determination of urine organic acids revealed elevation of 3-hydroxy-2-methylbutyryic (48 mmol/mol creatinine; CV 5–12) and tiglylglycine (44 mmol/mol creatinine; CV <5). HSD10 activity in fibroblast was 0.5 nmols/mg protx min (C.V. 1.4 ± SD 0.43) and a new missense mutation was detected in *HSD17B10* gen (c.628 C > T; p.P210 S) [7]. The mother was confirmed to be a carrier. Despite low isoleucine diet, the global outcome was very poor. At 3 years the child was unable to walk due to severe motor dyspraxia and choreoathetosis. Abnormal ocular movements and severe cognitive delay were present. At 5 years of age, the patient died of pneumonia leading to sepsis, multi-organ failure, and mitochondrial dysfunction (hyperlactacidemia, abundant Kreb's cycle metabolites in urine). Autopsy was not authorized.

CSF samples

CSF samples from our patient and controls were obtained by lumbar puncture as previously described [8]. First ten drops were used for routine cytochemical/microbiological studies and the rest immediately stored at −80°C until further analysis. The study of controls was performed in 30 subjects (age range: 21 days–5 years; average: 0.9 years, 16 boys, 14 girls) whose CSF samples were submitted under suspicion of central nervous system infection. Exclusion criteria were diagnosis of viral or bacterial meningitis, a chronic neurological condition, hyperproteinorrachia, and hematic or xanthochromic CSF.

Biogenic amine metabolites (3-orthomethyldopa, 3-methoxy-4-hydroxyphenylglycol, HVA, 5hydroxytryptophan, and 5-HIAA) and pterins (neopterin and biopterin) were analyzed by HPLC with electrochemical and fluorescence detection. Results were compared with our reference values [8].

Western blot analysis was performed for each protein (BDNF, Aβ, AS). Twenty μL of CSF was loaded on to the gel and proteins were separated on 10% sodium dodecyl sulphate-polyacrylamide gels and transferred to polyvinylidene difluoride (PVDF) membranes (Amersham™ Hybond™–ECL; GE Healthcare). Membranes were incubated in TBST buffer (0.02 M Tris-base, pH 7.6, 0.8% NaCl, 0.1% Tween 20) supplemented with 5% dried skimmed milk for 60 min to block non-specific binding. Anti-BDNF extracellular loop (1 : 500; Santa Cruz Biotechnology ®), anti-Aβ (1 : 500; Santa Cruz Biotechnology ®) and Anti AS (1 : 500; Santa Cruz Biotechnology®) antibodies were added, and the preparations were incubated at 4°C overnight. The membranes were washed three times with TBST buffer and then incubated with appropriate anti-rabbit (1 : 3,000; Promega®) or anti-mouse (1 : 5,000; Promega®) IgG secondary antibodies at room temperature for 1 h. The blots were then washed six times with TBST and prepared with ECL (Pierce® ECL Western Blotting Substract; Thermo Scientific) for developing. Relative levels of each protein were quantified by measuring optical densities (OD) of the corresponding bands with Quantity One® V 4.3.1 software.

CSF total protein concentration was measured by standard automated procedures in an Architect ci8200 analyzer (Abbott, USA).

Samples were obtained in accordance with the Helsinki Declaration of 1964, as revised in 2000. The ethical committee of the Hospital Sant Joan de Déu approved the study. Statistical analysis (linear regres-

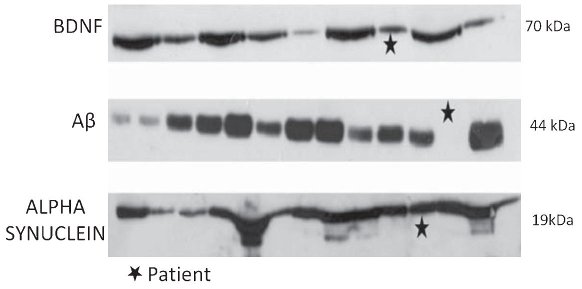

Fig. 1. Western blots of proteins in the patient and in controls.

sion; significance level: $p < 0.05$) was performed with the SPSS 19.0 program.

RESULTS

In our patient, CSF 5-hydroxyindolacetic acid concentration was low (133 nmol/L; C.V. 170–490) whereas dopamine metabolites were within normal limits (HVA: 360 nmol/L; C.V.: 344–906). BDNF, Aβ, and AS were detected in the CSF of the patient and controls, at the expected molecular weight (Fig. 1). CSF total protein concentration values in patients and controls were within normal limits according to different age ranges [9]. Linear regression showed no correlation between age and any of the studied proteins (Aβ: p:0,1; BDNF: p:0,2; AS: p:0,1). The expression of BDNF and AS in the CSF of our patient showed low values with respect to the control group, particularly when compared with age-matched controls (Fig. 2.). The expression of Aβ was undetectable in the patient whereas it was clearly present in all subjects of the control group. Given its negativity, it was repeated twice in two CSF samples (at 3 and 4.5 years), disclosing the same result.

DISCUSSION

HSD10 deficiency is a neurodegenerative disease with a complex pathophysiology. Furthermore, HSD10 is a protein that may mediate the neurodegeneration of AD through its apparent capacity to bind Aβ (1). Due to this affinity, we hypothesized that CSF Aβ concentration in patients with HSD10 deficiency could be abnormal and contribute to the neurobiology of this disorder. The most striking feature of this study is the lack of expression of Aβ in this patient in several samples obtained at different ages.

HSD10 catalyzes the conversion of 2-methyl-3-hydroxybutyryl-CoA to 2-methylacetoacetyl-CoA in the isoleucine degradation pathway, and is active against a broad range of substrates in diverse pathways such as steroid metabolism, $GABA_A$ receptors, and the oxidation of other substrates (hydroxyacyl-CoAs, 2-methyl-3-hydroxyacyl-coas) [1, 2]. Consequences of HSD17B10 gene mutations seem to be unrelated to accumulation of toxic metabolites in the isoleucine pathway but rather are caused by a non-enzymatic effect triggering mitochondrial disintegration and apoptosis [4].

Several studies indicate that mitochondria plays an important role in the development of AD, and this effect could be caused by a direct interaction of Aβ with MHBD (2-methyl-3-hydroxybutyryl-CoA dehydrogenase) [5–7]. HSD10 has a unique loop D (residues 95–114) that binds Aβ [1]. In AD brain tissue, Aβ accumulates in mitochondria. Conversely its concentration is low in CSF. HSD17B10 gene mutation in our patient could have modified this binding and promote post-synaptic Aβ trapping, thereby explaining the apparent absence of Aβ in the CSF.

Little is known about the neurobiology of Aβ in childhood. Recent studies suggest that Aβ and its precursor protein (AβPP) may play important roles in development such as promoting synapse elimination [10, 11], pruning neurites [12], and restricting mature forms of LTP in glutamatergic synapses [13]. In the rodent hippocampus, Aβ has a maximum expression during the period of most intense synaptogenesis and synaptic elimination [14]. Therefore, persistently low synaptic levels of Aβ in our patient could have impaired synaptic balance and glutamatergic transmission. Furthermore, increased post-synaptic Aβ levels could have disrupted synaptic plasticity and promoted apoptosis [15–18].

BDNF is associated with dendritic growth, synaptic transmission [19], and the development of the GABAergic and monoaminergic system [20]. In our patient, BDNF low expression argues in favor of low dendritic density and support reduced CSF concentration of 5-HIAA, as this neurotrophin regulates serotonin system development. Concerning AS, this is a presynaptic protein that binds to the SNARE complex [21] and is involved in regulation of vesicle pools. In fact, its deletion causes a reduction in the reserve pool size [22], leading to impaired long-term potentiation and synaptic plasticity. Synaptic accumulation of AS has been related to dopaminergic loss [23]. In our patient AS showed very low expression which perhaps might explain why CSF dopaminergic metabolites were not reduced as expected at high AS synaptic levels.

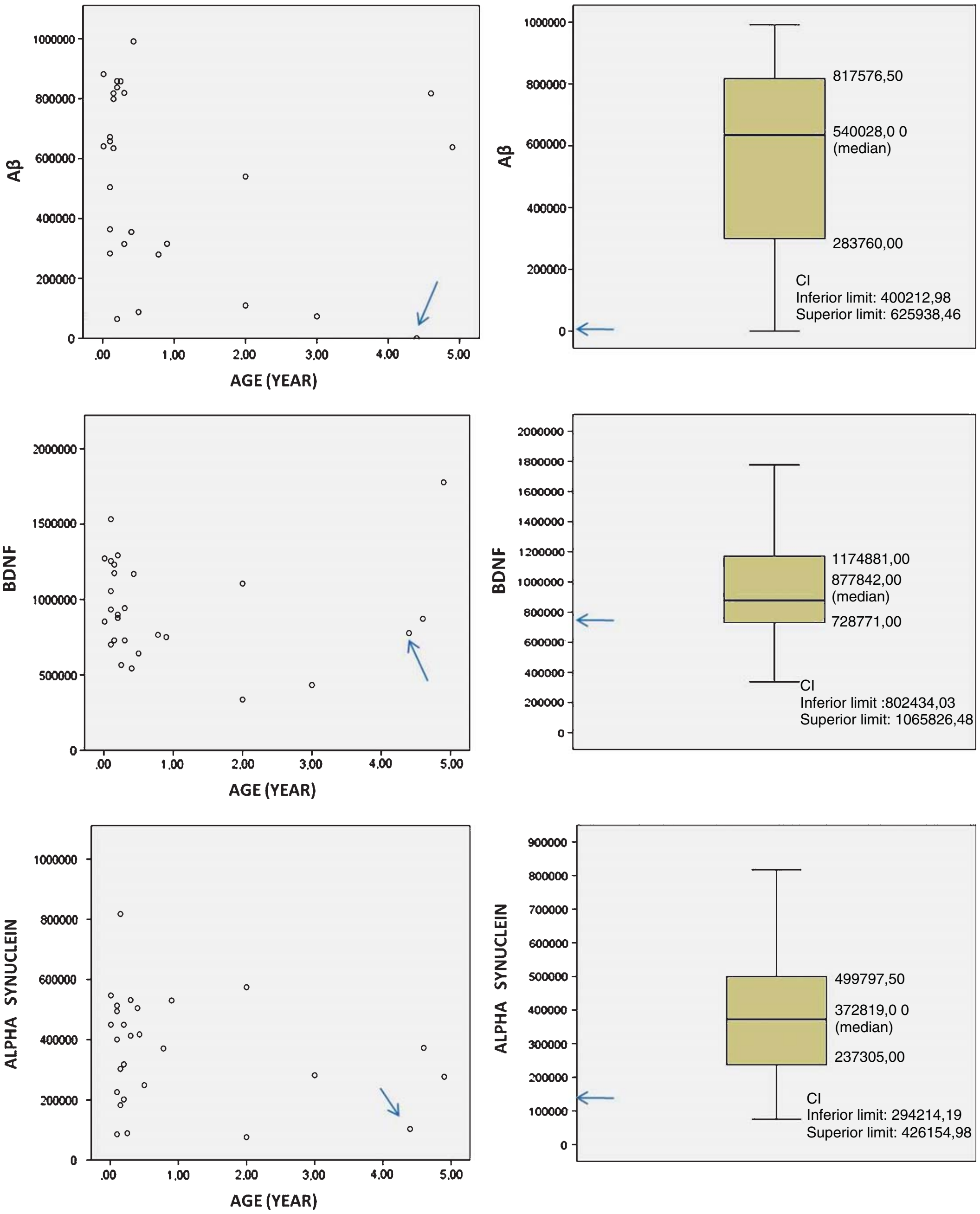

Fig. 2. Distribution of Aβ, BDNF and AS values in the patient (arrows) and in controls. Units on the left are optical densities. CI: confidence interval.

In summary, we report a patient with HSD10 deficiency, undetectable CSF Aβ expression, and low BDNF and AS levels, which probably disrupted critical developmental functions, contributing to impaired synaptic plasticity, low serotoninergic transmission and apparently preserved dopaminergic function.

Confirmation of these findings in other patients would help elucidating mechanisms of synaptic dysfunction in this disease, and highlight the need for further studies to better understanding of the role of Aβ in both early and late periods of life.

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