

Association of metreleptin treatment and dietary intervention with neurological outcomes in Celia's encephalopathy

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ABSTRACT

Celia's encephalopathy (progressive encephalopathy with/without lipodystrophy, PELD) is a recessive neurodegenerative disease that is fatal in childhood. It is caused by a c.985C>T variant in the *BSCL2*/seipin gene that results in an aberrant seipin protein. We evaluated neurological development before and during treatment with human recombinant leptin (metreleptin) plus a dietary intervention rich in polyunsaturated fatty acids (PUFA) in the only living patient. A 7 years and 10 months-old girl affected by PELD was treated at age 3 years with metreleptin, adding at age 6 omega-3 fatty acid supplementation. Her mental age was evaluated using the Battelle Developmental Inventory Screening Test (BDI), and brain PET/MRI was performed before treatment and at age 5, 6.5, and 7.5 years. At age 7.5 years, the girl remains alive and leads a normal life for her mental age of 30 months, which increased by 4 months over the last 18 months according to BDI. PET images showed improved glucose uptake in the thalami, cerebellum, and brainstem. This patient showed a clear slowdown in neurological regression during leptin replacement plus a high PUFA diet. The aberrant *BSCL2* transcript was overexpressed in SH-SY5Y cells and was treated with docosahexaenoic acid (200 μ M) plus leptin (0.001 mg/ml) for 24 hours. The relative expression of aberrant *BSCL2* transcript was measured by qPCR. *In vitro* studies showed significant reduction (32%) in aberrant transcript expression. This therapeutic approach should be further studied in this devastating disease.

KEYWORDS: BSCL2, PELD, neurodegeneration, recombinant human leptin, DHA, PET

INTRODUCTION

In 2013, our group described a new pediatric neurodegenerative syndrome (ref. 1). This syndrome, termed progressive encephalopathy with/without lipodystrophy or PELD (MIM: # 615924) is also known as Celia's encephalopathy. It has a fatal prognosis and is caused by homozygosity or compound heterozygosity of a c.985C>T variant in the *BSCL2* gene (NM_001122955.3). To date, 7 cases have been reported (ref. 1,2), with 6 dying before they were 9 years old (mean, 7 years; range, 6–8 years) as a consequence of the neurodegenerative process. During early infancy, psychomotor development is normal, but individuals with PELD show signs/symptoms of developmental delay around age 3 years. Subsequently, they unfailingly begin a process of rapid neurological regression and, less than one year later, they present with severe encephalopathy (Supplemental Video 1).

Studies by our group have shown that the c.985C>T variant leads to aberrant splicing, causing the loss of exon 7 of the *BSCL2* gene (ref. 1). This leads to the production of an aberrant seipin protein. Seipin encoded by a transcript without exon 7 tends to form macroaggregates that cause endoplasmic reticulum stress, activating the unfolded protein response and leading to neuronal death (ref. 3).

Here we report the unexpectedly long survival of the only living patient with Celia's encephalopathy, who as of January 2017 was 7 years and 6 months old. This patient was treated with recombinant human leptin and with dietary polyunsaturated fatty acids that could have some beneficial effects on neurodegeneration in terms of slowing down the process. Our *in vitro* studies in SH-

SY5Y cells showed that leptin plus docosahexaenoic acid (DHA) significantly reduced the expression of the aberrant *BSCL2* transcript, suggesting a possible mechanism of action for this treatment.

SUBJECT AND METHODS

The regional IRB approved this study, which was conducted according to the ethical guidelines of the Helsinki Declaration. The patient's parents gave informed written consent for the subject's participation in the study and for the publication of clinical, imaging and genetic information.

Subject

A girl was diagnosed with PELD, which was caused by the c.[509_513del];[985C>T] variants in the *BSCL2* gene (<https://databases.lovd.nl/shared/individuals/00002973>; <https://databases.lovd.nl/shared/individuals/00002973>, patient ID:00002973), when she was 18 months old.

Therapeutic intervention

Leptin treatment: Due to metabolic and hepatic complications (Table 1), treatment with recombinant human leptin (metreleptin) was started when the patient was 3 years old at a dose of 0.03 mg/kg per day subcutaneously. The dose was titrated according to weight gain and according to the response of the metabolic and hepatic parameters (Table 1).

Dietary intervention: Our *in vitro* data showed that a polyunsaturated fatty acid emulsion reduced the expression of the *BSCL2* aberrant transcript by around 50% in the

preadipocytes of other patient suffering from PELD (ref. 4). In addition, supplementation with exogenous oleic acid was found to increase the motility of zebrafish that overexpressed seipin N88S (ref. 5). Accordingly, starting when she was 6 years old, **the patient began a oral diet with natural food. The diet composition was assessed with the aid of a Registered Nutritionist by 24 hr recall questionnaires in each visit and 3 day food records. Results of nutritional calculations, using the Odimet® program (www.odimet.es) (mean +/- standard deviation) were: 1725 ± 6.2 Kcal [55.1±1.7% carbohydrates, 17.7± 1.5 % proteins, and 30.0 ± 2.0 % fats; being saturated fat 8.77 ± 0.72 g (C14:0, 0.58 ± 0.11 g; C16:0, 5.99 ± 0.60 g; C18:0, 1.28 ± 0.10 g), monounsaturated fat 28.93 ± 1.30 g (C16:1, 1.14 ± 0.03 g; C18:1 25.42 ± 0.79 g), polyunsaturated fat 14.36 ± 1.33 g (C18:2 6.62 ± 2.70 g, C18:3 3.66 ± 1.65 g, C>20 3.35 ± 1.78 g, docosahexaenoic acid (DHA) 1.52 ± 0.22 g, eicosapentaenoic acid (EPA) 0.85 ± 0.20 g), and cholesterol 230,33 ± 65.19 mg]. The girl also took a daily 0.57 g omega-3 fatty acid supplement: EpaDhax Kids 550 capsules Epadhax S.L., Boiro, A Coruña, Spain) t.i.d., each capsule contained 0.19 g of omega-3 fatty acids (EPA 0.09 g, DHA 0.39 g).**

PET and MRI studies

Standard 18F-FDG PET and volumetric MRI brain studies were performed. PET was performed based on the European Association of Nuclear Medicine standard clinical protocols for paediatric examinations. Brain MRI was performed under anaesthesia using a 1.5-Tesla magnet (Siemens Magnetom) with volumetric T1-weighted TSE, axial and coronal T2-weighted TSE, axial T1-weighted IR, T2 GRE and DWI (b values 0,

1000). PET and MRI scans were performed at the following times: just prior to the initiation of metreleptin treatment at age 3 years; at age 5 years; at age 6 years and 4 months; and at age 7 years and 6 months. All PET images were co-registered to MRI in order to combine functional and anatomical information in a common reference image. Fused PET/MRI images of the patient were obtained using a mutual information approach (Statistical Parametric Mapping, Wellcome Department of Cognitive Neurology, London, UK). PET images were evaluated longitudinally by prior spatial normalization to a common reference template in order to obtain quantitative comparisons of the same anatomical region along the PET images. The metabolism in the temporal and occipital regions, thalami, caudate and putamen nucleus, cerebellum and brainstem was compared longitudinally along the PET images using the frontal cortex as a reference.

Electroencephalogram (EEG)

EEGs were recorded digitally using 21 electrodes placed according to the International 10-20 System (Nihon Kohden Europe GmbH, Rosbach, Germany).

Battelle Developmental Inventory Screening Test (BDI)

The screening version of the BDI (ref. 6) includes five domains: personal-social; adaptive; fine and gross motor; communication; and cognitive. Using these domains, the patient's developmental age was calculated when she was 24, 36, 53, 64, 72, 77, and 90 months old.

Biochemistry

Fasting serum samples were analysed to determine the levels of glucose, triglycerides, total cholesterol, low-density lipoprotein-cholesterol (LDL-c), high-density lipoprotein-cholesterol (HDL-c), leptin and insulin, as described previously (ref. 7). Alanine transaminase (ALT), aspartate transaminase (AST), and gamma-glutamyltransferase levels were determined with enzymatic methods on an ADVIA analyser (Siemens, Bayer Diagnostics, Tarrytown, NY, USA). DHA was measured by gas chromatography (Agilent 7890A, Supelco, Bellefonte, PA) before and during dietary intervention.

***In vitro* studies**

Plasmids: A plasmid that encoded wild type human seipin fused to a myc tag (6x myc-wt seipin pCS2+MT) was the generous gift of Dr. D. Ito (Keio University, Japan). The Myc-fused Celia seipin expression plasmid was described previously (ref. 1). The lentiviral plasmid containing aberrant seipin was generated as follows: Aberrant seipin cDNA was amplified by PCR using specific primers (forward: 5'-TAAGCAGGTACCTCTTTTTGCAGGATCCCATCGA-3'; reverse: 5'-TAAGCATCTAGAGCCTTGAATTCGCCCTTGAC-3'), and the amplification product was digested with the fast-digest restriction enzymes KpnI and XbaI (cat. FD0524 and FD0684, Thermo Fisher Scientific), purified and inserted into the pLenti-CMV-GFP-2A-Puro-Blank vector (cat. LV073, Applied Biological Materials Inc.). The full sequence was confirmed by sequencing.

Lentiviral transduction: The SH-SY5Y neuroblastoma cell line was the kind gift of Dr. Jesús Rodríguez-Requena (University of Santiago de Compostela, Spain). Cells were maintained in a 1:1 mixture of Ham's F12 (cat. N4888, Sigma-Aldrich) and

Eagle's Minimum Essential Medium (cat. M2279, Sigma-Aldrich) supplemented with 15% heat-inactivated foetal bovine serum (cat. 10270-106, Gibco), 1% GlutaMax-I™ (cat. 35050-061, Gibco), 100 units/ml penicillin, 100 µg/ml streptomycin (cat. 15140-122, Gibco), and 1% MEM non-essential amino acid solution (NEAA, cat. 11140050, Gibco). Cells were grown in a 5% CO₂ atmosphere in a humidified incubator at 37°C. The medium was replaced every 3 days, and the cells were sub-cultured once 90% confluence was reached. In all of the experiments, the cells were used at early passages (below P17). For lentiviral transduction, cells were seeded into 6-well plates (cat. 3516, Corning, Costar) at a density of 5×10³ cells per cm². Twenty-four hours after seeding, the medium was removed, and the cells were washed with phosphate buffered saline (PBS). Viral particles (aberrant seipin or the empty vector as a control; functional titres of >10⁹ transducing units/ml produced by Cyagen Biosciences (Guangzhou, China), were added at a multiplicity of infection (MOI) of 200 viral particles/cell to serum-free medium in the presence of Lentiblast A/B (1:100/1:1000, Oz Biosciences, Marseille, France). Serum was added 4 hours after initial infection (final infection volume, 1 ml). Twenty-four hours after infection, the medium was removed and the cells were washed with PBS. The cells were then cultured with SH-SY5Y medium. Puromycin dihydrochloride (2 µg/ml final concentration; cat. P8833, Sigma-Aldrich) was added to the cell culture medium every 2-3 days until resistant stable cells had grown. Cells were then grown routinely as described above.

DHA and leptin treatment: DHA (cat. 2534, Sigma-Aldrich) was dissolved in absolute ethanol (100 mg/ml) and then conjugated to fatty acid-free, endotoxin-free bovine serum albumin (BSA, cat. A8806, Sigma-Aldrich) at a fatty acid:BSA molar ratio of 4:1

(200 μ M DHA:50 μ M BSA). The solution was vortexed thoroughly for 2 minutes and incubated at 37°C for 2 hours with vortex mixing every 15 minutes. Stably transfected cells were seeded into 6-well plates at density of 10×10^3 cells per cm^2 and allowed to adhere for 2 days. Subsequently, the cells were incubated for 24 h with or without DHA 200 μ M conjugated to fatty acid-free BSA plus human leptin (0.001 mg/ml, cat. L4146, Sigma-Aldrich). The leptin dose was chosen based on the dose used in human treatment, assuming a distribution volume of 5 litres. Medium with 0.66% ethanol (v/v) and 50 μ M fatty acid-free BSA was used for untreated cells. Cells were harvested the next day. This experiment was repeated three times.

RNA extraction and retrotranscription: Total RNA was extracted from cells using Trizol (Invitrogen, Madrid, Spain) as per the manufacturer's instructions. The concentration and purity of each sample was determined by spectrophotometer (ND2000; Nanodrop). RNA samples were stored at -80°C until use. The RNA was reverse-transcribed using M-MLV reverse transcriptase (Invitrogen) as described previously (ref. 8).

Real-time RT-PCR: Specific primers and probes were designed by the Universal ProbeLibrary (Roche Diagnostics, Sant Cugat del Valles, Spain) and used in a LightCycler 2.0 (Roche Diagnostics) to determine the specific expression of the *BSCL2* transcript without exon 7. The reverse primer, 5'-AGCGATCATTGAGATCCACA-3', was used with probe #42 (CATCCAGC); the forward primer, 5'-TTTTCGGATGTAAACCTGAGC-3', exclusively hybridized with the union of exon 6 and exon 8 (amplicons, 96 nucleotides). Real-time RT-PCR conditions are available

upon request. The results were normalized to the *18S* gene using the $2^{-\Delta\Delta CT}$ method (ref. 9).

Statistical analysis

Real-time PCR analyses were performed in duplicate. Statistical significance was determined using a non-parametric Mann-Whitney test. Data are presented as means \pm SD with the statistical significance set at $p < 0.05$. All statistical analyses were performed using SPSS for Mac (release 22.0; SPSS, Chicago, IL, USA).

RESULTS

Case report since diagnosis until the onset of metreleptin treatment

The girl, who was 7 years and 10 months old in May 2017, appeared normal at birth. Starting when she was just a few months old (Table 2), the patient showed a generalized lipodystrophy, with well-defined musculature and hepatomegaly. From a neurological point of view, her psychomotor development was normal in terms of milestones, although with slight delays, and there was no evident regression in the first two years of life. At around 24 months, her gait stability was lower than expected for her age but without abnormal movements. At 3 years, there was some improvement in her language, but she showed slow development for her age (Table 2). At that time, a PET/MRI scan was performed that showed brain hypometabolism, which was reported in a previous publication (Figure 1A) (ref. 1). There was marked hypometabolism of the left thalamus at the level of the basal ganglia relative to the contralateral thalamus.

Clinical evolution since the onset of metreleptin treatment until 6 years of age

At 3 years, the patient had hypertriglyceridemia, hyperinsulinemia and hypertransaminasemia (Table 1), so treatment with metreleptin (Aegerion Pharmaceuticals, CA, USA) was started as compassionate use. This therapy rapidly improved the patient's plasma triglyceride concentrations, insulinemia and liver transaminase levels, and these remained acceptably reduced at the time of this report, despite plasma leptin levels were low when patient was 7 years old (ref. 10).

Although her growth was appropriate for her weight, and head circumference, which were in the normal range, as expected in Berardinelli-Seip syndrome, her height was in a higher percentile (Table 1).

In terms of neurological function, when the girl was 4 years and 11 months old, her parents reported that she was sporadically unstable. When she was 53 months old, her developmental age was only 34 months according to the BDI (Figure 2). When she was 5 years old, a second MRI/PET scan was performed, and there was clear deterioration compared to the MRI/PET conducted when she was 3 years old. Specifically, **in MRI**, both caudate nuclei and thalami had decreased in size. There was hypersignal in the white matter adjacent to the body of both lateral ventricles as well as in the region anterior to the head of both caudate nuclei. Likewise, there was a slight decrease in the volume of white matter in both outer capsules. On the other hand, PET images obtained 2 years after the onset of metreleptin treatment, when the patient was 5 years old, showed decreased metabolism in the occipital region, and the thalamic asymmetry had disappeared due to a decrease in metabolism in the right thalamus (Figure 1B). There was a very important decrease in metabolism in both caudate nuclei.

When the girl was 5 years and 4 months old, the parents reported that they had observed significant clinical worsening in the previous few months, with regression in all areas i.e. language, cognitive and behavioural areas (Table 2). Her developmental age as calculated using the BDI was 24 months; this represented a 10-month deterioration since the previous evaluation, which was performed when she was 4 years and 5 months old.

In vitro studies with SH-SY5Y cells overexpressing the *BSCL2* aberrant transcript

Our *in vitro* studies showed that 24-h treatment of cells with human leptin plus DHA significantly reduced the expression of the aberrant seipin transcript (an average 32% reduction in three independent experiments) (Figure 3).

Clinical evolution since dietary intervention until 7 years and 10 months of age

By the time the girl was 6 years old, her parents reported that she was unstable at times and fell easily (Table 2). At that point, a first EEG showed slow tracing but not the paroxysmal activity that is typical of epilepsy. Her developmental age at that time was 26 months. She was started on an oral diet that was rich in polyunsaturated fatty acids plus a daily omega-3 fatty acid supplement. When she was 6 years and 9 months old, MRI/PET was performed; this was 2 years after the previous MRI/PET. The MRI results showed that both the caudate nuclei and thalamus continued to decrease in size. On the other hand, a hyperintense signal was observed in the white matter adjacent to the bodies of both lateral ventricles as well as in the region in front of the heads of both caudate nuclei. Likewise, there was a slight decrease in the volume of white matter in both of the outer capsules with respect to the 2014 study, performed when the girl was 5

years old. The PET results (Figure 1C) showed a significant increase in glucose uptake in both thalami and in the brainstem.

The girl continued to fall, and her instability persisted. An EEG was performed when she was 6 years and 11 months old, and 31 myoclonic epileptic seizures were registered during the 3-hour EEG video. Treatment with levetiracetam (until 600 mg bid) was initiated, which reduced the number of seizures; however, valproic acid (250 mg bid) was added because myoclonus reappeared 2 months later. Subsequently, when the patient was 7 years and 3 months old, clonazepam (0.5 mg bid) was added due to a lack of control of the myoclonic seizures, and levetiracetam and valproic acid were withdrawn.

Currently, at the age of 7 years and 10 months, the patient does not have seizures, and the neurodegenerative process appears to have improved. Compared with the previous PET scan, the most recent PET scan (at age 7 years and 6 months) showed a reduction in glucose metabolism in the occipital region; slight improvement in both thalami, temporal lobes and brainstem; and clear improvement in the cerebellar region (Figure 1D). Compared with the previous MRI, the most recent MRI showed a decrease in the volume of the head of both caudate nuclei and a clear loss of white matter bilaterally in the external and internal capsules. The BDI showed a developmental age of 30 months (a 4-month improvement since the previous evaluation). The final EEG showed frequent bursts of spike activity, wave activity and spike-wave activity that were synchronous and asynchronous over both central-parieto-temporo-occipital lobes (Supplementary Figure 1S). That said, the girl has a reasonably normal quality of life

for her neurological age, goes to school (care for children with special needs) and interacts with her parents and younger brother. She can respond to simple commands and perform some simple tasks (Supp. material Video 2).

DISCUSSION

Celia's encephalopathy is a devastating, extremely rare neurodegenerative disease that leads inevitably to death in childhood (ref. 1). Starting when they are 3 or 4 years old, affected children progressively lose all neurological function, and this loss is accompanied by seizures that can barely be controlled with antiepileptic drugs.

In this study, we described the unique natural history of the only living patient (to the best of our knowledge) with Celia's encephalopathy. Currently, at age 7 years and 10 months, the girl shows obvious signs of encephalopathy, but she is not as affected as one might expect given the natural history of the other 6 reported cases, who died, on average, at age 7 years. If we compare the evolution of the onset of symptoms in this patient, i.e. the onset of psychomotor delays, neurological regression, seizures and death (Figure 4), it is clear that the period of developmental delay without neurological regression lasted longer (up to 5 years) than in the other reported cases, and the neurological regression appeared to be slower. In the same way, the appearance of myoclonic epilepsy was detected much later than expected in comparison to the other 6 cases.

As noted, this patient started early treatment with metformin in order to control her metabolic disturbances. Subsequently, at 6 years of age, her diet was modified by increasing the proportion of polyunsaturated fatty acids and adding

omega-3 supplementation. Before this intervention, functional impairment was evident in the cerebral PET images, especially in the basal ganglia, the temporal and occipital lobes and the cerebellum and brainstem. Interestingly, the last two PET scans, one when the patient was 6 years and 9 months old and one when she was 7 years and 6 months old, showed improvement in previously affected areas, except for the caudate and putamen nuclei and the occipital lobes. Most notably, both thalami showed increased 18-F-glucose uptake (+20%), and also the cerebellar regions and brainstem experienced a striking improvement.

Regarding higher function and motor skill development, the child showed gradual deterioration from age 2 years until the present, although the deterioration occurred at a considerably slower rate than in the other cases. Strikingly, she showed modest improvement over the last 18 months according to the BDI.

In light of these results, it is tempting to speculate that the combination of metreleptin plus increased dietary polyunsaturated fatty acid levels positively affected the patient by slowing the neurodegenerative process. There is a reasonable body of scientific evidence suggesting that leptin has a beneficial effect on central nervous system function (ref. 11,12) and on some neurodegenerative disorders, like Alzheimer's disease (AD) (ref. 13,14). In addition to its well-known effects on the regulation of food intake and on energy expenditure in the hypothalamus (ref. 15), leptin can affect neurogenesis, synaptogenesis, neuronal excitability, axon growth and dendritic morphology and can have neuroprotective effects (ref. 12,16-21). Moreover, leptin regulates the synapse morphology of hippocampal neurons (ref. 22) and modulates the

development of oligodendroglial cells (ref. 23), which may contribute to structural changes in grey matter.

In addition, leptin may help prevent neuronal death in neurodegenerative disorders (ref. 12). For example, in transgenic mice models of AD, leptin replacement therapy (LRT) results in significant cognitive improvement (ref. 13) and reduces the brain amyloid-beta peptide (A β) load (ref. 14). The neuroprotective effects of leptin involve the following: activation of the JAK2-STAT3, AKT and MAPK/ERK signalling pathways (ref. 24); inhibition of apoptotic cell death and improved cell survival via the regulation of apoptotic enzymes; protection against glutamatergic cytotoxicity; defence against oxidative stress via expression of the membrane antioxidant MnSOD and stabilization of mitochondrial membranes; and the promotion of hippocampal progenitor cell proliferation (ref. 12). Interestingly, high circulating leptin levels in humans are associated with a reduced incidence of dementia (ref. 19,25), and leptin signalling seems to be dysregulated in the brains of AD patients (ref. 26). Notably, in leptin deficiency disorders such as lipodystrophy and congenital leptin deficiency (CLD), LRT results in different degrees of improvement in some brain functions. For example, grey matter density is increased in diverse brain regions in CLD that is treated with leptin, and improvements in neurocognition have been reported in isolated cases (ref. 27). In lipodystrophic patients, LRT increases food-related neural activity in the orbitofrontal cortex and suppresses activity in the amygdala, hippocampus, insula, caudate and putamen (ref. 11).

Regarding dietary intervention, there are some studies that support the role of dietary polyunsaturated fatty acids, particularly DHA, on brain development in children (ref. 28-31), and it may also have beneficial effects on neurodegenerative diseases such as AD, Parkinson's disease, attention deficit hyperactivity disorder, autism and traumatic brain injury (ref. 32-36), although some results are controversial (ref. 37,38). DHA is the most abundant omega-3 long-chain polyunsaturated fatty acid in nerve cells, and it largely determines the structural and physicochemical properties of the plasma membrane, including membrane viscosity, lateral mobility, phase separation and microdomain segregation, conformational transitions and the stability of membrane proteins and lipid-protein and protein-protein interactions (ref. 39). DHA modulates neurogenesis, synaptogenesis and neurite outgrowth; refines synaptic connectivity; controls neurotransmitter release; plays a role in memory consolidation processes (ref. 29,40,41); and is protective against trauma or ischemic insults. All of these processes are highly relevant to understanding and treating any neurological disorder. Therefore, dietary modifications and supplementation may have neuroprotective and therapeutic effects (ref. 42). However, we caution that too much omega-6 during neural development in children negatively affects neurite growth and may contribute to poor neurodevelopment (ref. 43). In addition, altering the omega-6:omega-3 ratio during early life may induce developmental changes in brain connectivity, synaptogenesis, cognition and behavior (ref. 44).

Although we are aware that this could be a case of variable expression, as happens in other Mendelian diseases, the natural history of the other 6 cases was remarkably consistent. Also it is possible that anti-epileptic therapy could have some

effect on the BDI results. However, the clear improvement in glucose uptake in several brain regions was not expected in a neurodegenerative disease.

Our *in vitro* results strongly suggested that the combination of human leptin plus DHA has some effect on the splicing rate of the aberrant *BSCL2* transcript, at least in cells in which it is overexpressed. Although we do not know the precise molecular mechanisms underlying this finding, other authors have reported that DHA differentially modulates the expression of transcripts both in brain (ref. 39) and in other tissues (ref. 45).

In summary, our results suggest that treatment with recombinant human leptin plus a dietary intervention might slow down the fatal natural course of Celia's encephalopathy.

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Conflict of Interest

D. A-V received an honorarium as an expert advisor from Aegerion Pharmaceuticals; the other authors have no disclosures.

Supplementary information is available at European Journal of Human Genetics' website.

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TITLES AND LEGENDS TO FIGURES

Figure 1 Standard 18F-FDG PET brain of a girl with Celia's encephalopathy (A) at age 3 years (before therapeutical intervention); (B) at age 5 years; (C) at age 6 years and 4 months; and (D) at age 7 years and 6 months. A. The images show hypometabolism at the level of both temporal lobes that is symmetrical in both hemispheres and an area of lower metabolic activity in the occipital region that is more evident in the medial planes, cerebellum and brainstem. The remaining areas of the cerebral cortex did not show significant metabolic alterations at that time. There is marked hypometabolism of the left thalamus relative to the contralateral thalamus at the level of the basal ganglia. B. The images show that metabolism in the occipital region is decreased by -17.9%. The thalamic asymmetry disappeared due to a loss of metabolic activity in the right thalamus of -9.9%, while the left thalamus remained unchanged (+1.3%). There were no significant changes at the temporal lobe level, but there was a very important decrease in metabolism in both caudates nuclei (-35.5%) plus a slight decrease in metabolism in both of the putamen nuclei (-9.9%). Very low glucose metabolism is evident in the brainstem and cerebellar regions. C. The images showed slight metabolic recovery in the occipital region (+9.6%) and a significant increase in glucose uptake in both the thalamus (+22.2%) and brainstem (+25%). There were no significant changes at the temporal level, although there was a slight decrease in metabolism in both caudates (-6.5%) and a decrease in uptake in both putamen nuclei (-12.8%). D. Compared to the images in (C), there was a reduction in glucose metabolism in the occipital region (-14%), slight improvement in both thalami (+6%) and temporal lobes (+5%), no changes in the brainstem (+2.5%) or in the caudate and putamen and clear improvement

in the cerebellar region. E. Standard 18F-FDG PET brain in a healthy 12-year-old girl (Control 1). F. Standard 18F-FDG PET brain of a newborn at age 20 days (Control 2); the image shows marked metabolism in the brainstem.

Figure 2 The mental age of a girl with Celia's encephalopathy as calculated by the Battelle Developmental Inventory Screening Test (BDI). The BDI was performed several times along 5.5 years. The girl's mental age was always lower than her chronological age beginning when she was 2 years old. When she was 3 years old, her mental age was 2 years; this was just before she started metreleptin treatment. Her mental age progressed slowly, and she was found to have a mental age of 34 months when she was 4 years and 5 months old; subsequently, she worsened, showed a mental age of 2 years when she was 5 years and 4 months old. When she turned 6 years old, the patient began a dietary intervention with omega-3 fatty acid supplements. Her mental age started to improve and was 30 months when she was 7.5 years old.

Figure 3 The effect *in vitro* of docosahexaenoic acid (DHA) plus leptin treatment on SH-SY5Y neuroblastoma cells that overexpress the aberrant *BSCL2* transcript. After 24 hours of treatment, the relative expression of aberrant transcript was significantly reduced by an average of 32% in three independent experiments. Relative expression of the aberrant *BSCL2* transcript. * $p < 0.01$ vs. vehicle. All samples were analysed in duplicate, $n = 3$. Results were normalized using the *18S* gene.

Figure 4 The natural history of Celia’s encephalopathy in the 7 patients reported to date. Panel A shows the 6 patients who died in childhood. Developmental delays started at 2 years of age and extended until age 3.5 years (purple). Neurological regression was subsequently apparent (red), and seizures (orange) began before age 4 years. The patients died at an average age of 7 years (black). Panel B shows the disease time-course of the patient who is currently alive. Development delays started at 2 years of age and extended until age 5 years (purple); subsequently, neurological regression was apparent (red), and seizures appeared at 6 years and 11 months of age (orange). Therapeutic intervention is represented by arrows: metreleptin (green), dietary intervention (blue) and antiepileptic drugs (black).

SUPPLEMENTARY MATERIAL

Figure 1S. An electroencephalogram (EEG) of the patient when she was 7 years and 4 months old showed slow background activity and two generalized discharges consisting of spike-waves and degraded spike-wave complexes of 2.5–3.0 Hz frequency with up to 550 μ V amplitude and a predominance over the frontal lobes. The first EEG (A) shows no evidence of clinical seizures, the second EEG (B) is characterized clinically by the presence of a rhythmic flicker.

Video 1 The first reported case of Celia’s encephalopathy, now deceased, at 5 years of age.

Video 2 The patient with of Celia’s encephalopathy who is reported in this manuscript when she was 7.5 years old.

EJHG-Tube-Video

LEGENDS FOR VIDEOS

Video 1. The first patient who was diagnosed with PELD died in 2012 when she was 8 years old. In the video, the patient is 5 years old, and severe encephalopathy is evident. This patient was unable to walk or sit unsupported, had lost all motor skills and social, language and cognitive skills, and had sporadic unintentional movements of the mouth and extremities.

Video 2. The patient described in this study at age 7.5 years. The patient can walk and run without help, although her gait is ataxic. She maintains perfect visual contact with the investigator and follows the commands. She is very affectionate with her parents, and she obeys their directions.

Tube-Video Blurb

In this video we present the description of the natural history of a young girl suffering Celia's encephalopathy (progressive encephalopathy with/without lipodystrophy) who was treated with recombinant human leptin (metreleptin) and dietary intervention supplemented with omega-3 fatty acids. Our results suggest that this treatment might slow down the fatal natural course of Celia's encephalopathy.