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CLINICAL CASE REPORT

Evolution of the retinal function by flash-ERG in one child suffering from neuronal ceroid lipofuscinosis CLN2 treated with cerliponase alpha: case report

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Abstract

Introduction Neuronal ceroid lipofuscinoses (CLN) are neurodegenerative disorders among the most frequent, inherited as an autosomal recessive trait. Affected patients can present with progressive decline in cognitive and motor functions, seizures, a shortened life span and visual deficiency. CLN2 is one of the rare CLN that benefits from treatment by cerliponase alpha an enzyme replacement therapy. Preliminary results on treated animal models have shown delayed neurological signs and prolonged life span. However, cerliponase alpha did not prevent vision loss or retinal degeneration in those animal models. Cerliponase alpha has currently been delivered to a few CLN2-affected patients. We report the case of one patient suffering from CLN2 treated with intracerebroventricular infusions of cerliponase alpha 300 mg every two weeks. Evolution of his retinal function was assessed by three successive flash-ERG and flash-VEP

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recordings throughout his treatment over a 4-year period.

Results Before treatment at the age of 4 years 5 months, patient's retinas were normal (normal fundi and normal flash-ERG). After 29 infusions at the age of 6 years 10 months, a-wave combined response was absent, while cone and flicker responses were normal. After 80 infusions at the age of 8 years 9 months, a-wave cone response was absent with b-wave peak time increased, and no combined response.

Comments Despite treatment, our patient's retinas showed a progressive abnormal and inhomogeneous function. Rods function was altered first, then the scotopic system and afterward, the cones. This result differs from those recorded in animal models. The relative preservation of cone functioning for a while could not be unequivocally attributed to enzyme replacement therapy as we lack comparison with the evolution of flash-ERGs recorded in untreated subjects.

Keywords Neuronal ceroid lipofuscinosis CLN2 · Cerliponase alpha · Human tripeptidyl peptidase-1 (TPP1) · Evolution of retinopathy in CLN2 · Flash-ERG and flash-VEP in CLN2

Abbreviations

cd	Candela
cd/m^2	Candela per meter square
cd.s/m^2	Candela.second per meter square
CLN	Neuronal ceroid lipofuscinoses
CLN2	Late infantile neuronal ceroid lipofuscinosis (Jansky–Bielschowsky)
CLN2	*CLN2* Gene
ERG	Electroretinogram
VEP	Visual evoked potential

Introduction

Neuronal ceroid lipofuscinoses are neurodegenerative disorders among the most frequent, inherited as an autosomal recessive trait. Affected patients can present with progressive decline in cognitive and motor functions, seizures, a shortened life span and visual deficiency [1, 2]. CLN correspond to an abnormal storage of autofluorescent ceroid lipopigments within nerve cell lysosomes and visual system cells resulting in progressive neural dysfunction and retinal degeneration [1–3]. Different hypotheses have emerged to explain the lipopigment accumulation: endolysosomal dysfunction, lysosomal autophagy dysfunction, oxidative damage of intralysosomal proteins and others [3]. Recently, a better comprehension of lysosome roles, in particular their intralysosomal functions, was a step toward emerging therapeutic strategies for various CLN [2, 4]. CLN2 is the late infantile neuronal ceroid lipofuscinosis (Jansky–Bielschowsky) caused by mutations in *CLN2* gene [5]. Its onset is between 2 and 4 years of age. Symptoms include neurodevelopmental deterioration, seizures, ataxia, hypotonia, loss of language. Rapid visual impairment occurs with progressive vision loss between 5 and 6 years of age as a result of retinopathy and optic atrophy. Death happens in late childhood or early adolescence [6].

Treatment involves palliative cares [7]. Recently, enzyme replacement therapy with cerliponase alpha has been delivered to few affected patients in USA and Europe since 2017 [8–10]. Cerliponase alpha is a replacement enzyme of the human tripeptidyl peptidase-1 (TPP1) which is deficient in CLN2. It has been developed by BioMarin Pharmaceutical Laboratory [11]. This enzyme was first tested in mouse and canine models by administration of cerliponase alpha delivered intracerebroventricularly [12–14] because it does not cross the blood–brain barrier [15]. Stabilization of neurological regression and prolonged life span were observed. However, cerliponase alpha did not prevent vision loss or retinal degeneration in these animal models [16].

Delivered to CLN2 patients, cerliponase alpha was well tolerated. However, complications such as infections or dysfunction of the intracerebroventricularly system have been reported [17]. Significant attenuation of neurodegeneration has been observed in comparison with natural evolution [18, 19]. Retinal function evolution in these treated patients was not described.

We report the case of one child suffering from neuronal ceroid lipofuscinosis-2 (CLN2) diagnosed at 5 years of age and treated with intracerebroventricularly infusions of cerliponase alpha. We followed his retinal function by successive flash-ERG and flash-VEP recordings throughout his treatment.

Subject

The patient we describe had frequent falls with myoclonic seizures and loss of speech around the age of three years. The diagnosis of CLN2 was made just before the age of 5 years as he was only able to stay seated and walk with assistance. He had persistent myoclonic seizures despite combination of appropriate antiseizure treatment. Ocular pursuit was normal with no nystagmus. His fundi were normal. After placement of an Ommaya reservoir, he has received an intracerebroventricular dose of 300 mg of cerliponase alpha every two weeks since he was 5.5-year old [20, 21].

His visual function was assessed by flash-ERG and binocular flash-VEP recordings at diagnosis (age 4 years and 5 months), after 29 infusions of cerliponase alpha (age 6 years and 10 months) and after 80 infusions (age 8 years and 9 months). At this third recording session, our patient was very tired as the infusion sessions last more than 4 h. He was able to open his eyes but unable to have contact. He could not speak and only emitted some sounds. He had some dystonic movements in his four members. His visual acuity evaluation or fundus examination was not possible.

Materials and methods

The electrophysiological device used was a MonColor system (Métrovision, 59 Pérenchies, France) for the first recording and a Nihon Kohden system (92, Le Plessis-Robinson France) and for the two others.

Flash-ERG recordings

The child was never sedated. His pupils were not dilated given his physical conditions. Child size skin electrodes were used (Comepa, Neurocom, 93 Bagnolet, France) [22, 23]. Two active electrodes were applied on each inferior eyelid. The forehead was used as reference electrode site, and the two earlobes were connected to the ground. After a short light adaptation to a white background (30 cd/m^2), two light-adapted flash-ERG responses were recorded to standard flash (3 cd.s/m^2) (i.e., bright flash): cone response and flicker response (30 Hz). After 10 min of dark adaptation, the combined response was recorded to standard flash. This response is not equivalent to ISCEV standard mixed response, but it was not possible to make the recording session last longer. This combined response is dominated by the response of the scotopic system [24, 25].

Binocular flash-VEPs were recorded while both opened eyes were stimulated together. Cortical visual evoked responses (i.e., binocular VEP) were recorded with two active scalp electrodes placed on O2 and O1 according to the international 10/20 system [26]. The number of averages was 50. Two averages were performed to verify the reproducibility of each VEP. Background noise was recorded. Analysis time was over 750 ms to visualize the response adequately.

Results

ERG-VEP was always recorded in difficult conditions for the child, after the infusion session that lasts at least 4 h. Results were considered normal when between mean value ± 2SD.

Result values are given in Table 1.

Figure 1 shows that before diagnosis at the age of 4 years and 5 months, flash-ERG could be considered as normal according to child's and recording conditions. The cone-response waveform presented a normal a-wave. B-wave cone response presented a dual positive waveform. According to short light adaptation duration, this flash-ERG was considered normal. Flicker and combined responses were normal. After 29 infusions of cerliponase alpha at the age of 6 years and 10 months: cone and flicker responses were similar to those previously recorded and considered normal, combined a-wave amplitude was absent with normal combined b-wave amplitude but increased peak time. After 80 infusions of cerliponase alpha at the age of 8 years and 9 months, only two flash-ERG recordings could be interpreted due to the child's condition. Cone a-wave and cone b1-wave amplitudes were absent with normal cone b2-wave amplitude and peak time. Flicker response could not be interpreted and combined response was absent.

Figure 2 Binocular flash-VEP were recorded when both eyes were opened, on the two channels O2 and O1. Their amplitudes were abnormally increased at the age of 4 years and 5 months (45–50 μV peak to trough) and at the age of 6 years and 10 months (60–65 μV peak to trough). Binocular flash-VEP had abnormal waveforms

Table 1 Summary of flash-ERG and binocular flash-VEP wave amplitude (μV) and peak time (ms) values according to age in comparison with normal values with standard deviation. Ab-Nl = abnormal, Ampl = amplitude, Nl = normal, SD = standard deviation. Results were considered normal when between mean ± 2SD

Flash ERG	Cone response						Flicker resp	Combined-response			
	a-wave		b1-wave		b2-wave			a-wave		b-wave	
	Ampl μV	Peak time ms	Ampl μV	Peak time ms	Ampl μV	Peak time ms	Ampl μV	Ampl μV	Peak time ms	Ampl μV	Peak time ms
Nl subject	-8	18	17	37	—	—	20	-31	23	52	43
1 SD	5	4	6	12			6	7	3	12	4
CLN2 Child											
Cerliponase Alpha											
Before infusion	Nl	Nl	Nl	Nl			Nl	Nl	Nl	Nl	Nl
4Y 5m	-12	17	19	38	22	55	12	-30	25	62	49
After 29 infusions	Nl	Nl	Nl	Nl			Nl			Nl	↗
6Y 10m	-8	18	18	37	19	58	18	0	—	57	62
After 80 infusions	**0**	—	**0**	—	25	62	Not interpretable	**0**	—	**0**	—
8Y 9m											
	Binocular flash VEPs			**B- noise**							
	Waveform	Ampl peak to trough μV									
Nl subject		10 +/-5 μV									
Before infusion											
4Y 5m	Nl	>> 2-SD abnormally increased		**Ab-Nl**							
		45- 50 μV									
After 29 infusions											
6Y 10m	Nl	>> 2-SD abnormally increased		Not recorded							
		60-65 μV									
After 80 infusions											
8Y 9m	**Ab-Nl**	Nl		Nl							
		10-15 μV									

at the age of 8 years and 9 months, with amplitudes within normal limits for corresponding age. The background noise was abnormal and disturbed by high cortical electrical responses at the age of 4 years 5 months and nearly normal (isoelectric) at the age of 8 years and 9 months.

Comment

Weleber [27] and Quagliato et al. [28] reported altered flash-ERGs in all their CLN2 patients. Dozieres-Puyravel et al. [29] analyzed flash-ERG at diagnosis in 9 CLN2 patients whose mean age was 4 years and 4 months ± 5 months. Their visual assessments were normal. Out of those nine untreated patients, six had normal flash-ERG at diagnosis and three had responses with decreased amplitude without further details. No flash-ERG-VEPs were recorded on those children a few months later, making comparison with our patient's flash-ERG evolution not possible.

Flash-ERG and flash-VEP results were interpreted by experienced physicians in pediatric electrophysiology. They took into account the nonstandard light and dark adaptation durations. These nonstandard conditions were adapted to the child's condition and obligation to have correct results in the shortest time possible.

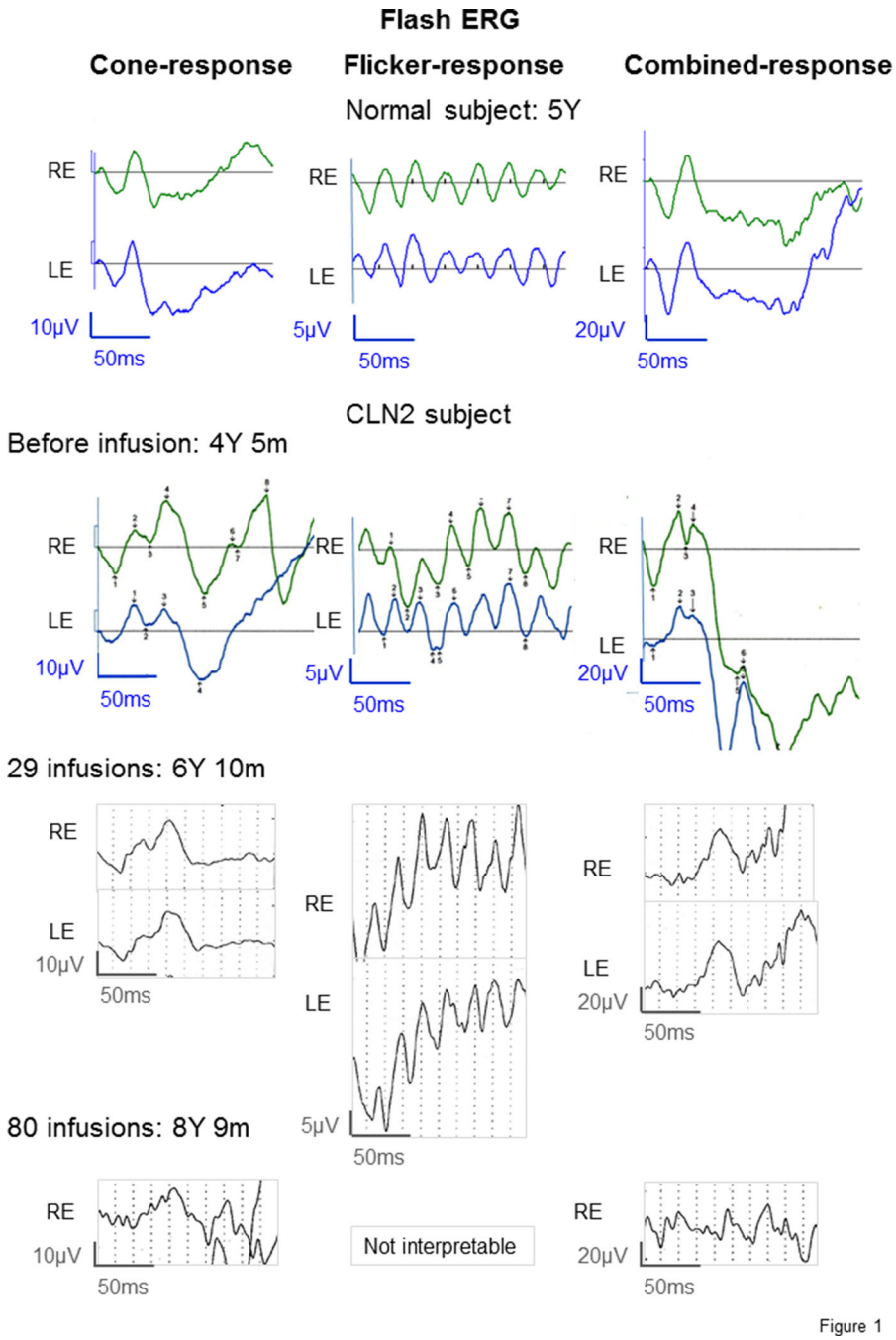

Fig. 1 Flash-ERG evolutions in our patient suffering from CLN2, before treatment by cerliponase alpha: normal-ERG taking into account the duration of adaptation to light. After 29 infusions, combined-ERG a-wave amplitude was null. After 80 infusions, photopic- and combined-ERG a-wave amplitudes were null and combined-ERG b-wave amplitude was null, i.e., combined ERG was no more discernible. 5Y = 5 years – 4Y 5 m = 4 years 5 months. RE = right eye, LE = left eye. Scales: on graph. ERG: (+) upward and (−) downward

Before diagnosis at the age of 4 years 5 months, flash-ERG cone response had a b-wave waveform differing from the normal one, with a dual positive b1- and b2-waves. These b1- and b2-waves are explained

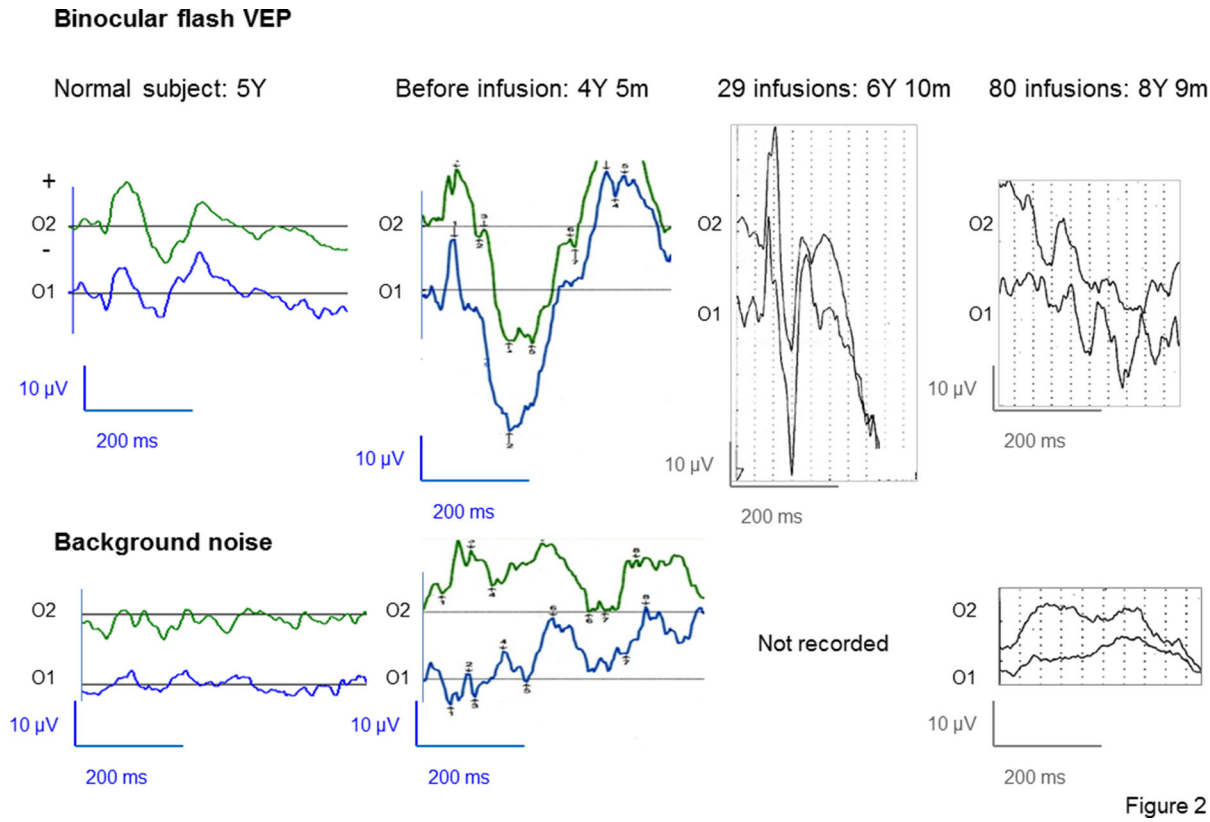

Fig. 2 Binocular flash-VEP evolutions in the same patient suffering from CLN2 recorded on two channels O2 and O1. Before treatment with cerliponase alpha and after 29 infusions, binocular flash-VEP amplitudes were abnormally increased (> 2 SD). After 80 infusions, binocular flash-VEP amplitudes were decreased with normalized background noise. Scales: on graph. VEP: (+) upward and (−) downward

by the short adaptation duration to light before cone-response recording, child's retinas being not fully adapted to light. Complete retinal light adaptation requires at least a 10 mn exposure to saturate the rod response fully. B1-wave corresponds to the normal cone b-wave of probably smaller amplitude. B2-wave probably corresponds to the response of the incompletely saturated rods. Taking those conditions into account, flash-ERG could be considered normal. Therefore, at diagnosis (4 years 5 months), normal fundi and normal flash-ERG confirmed that the child's retinae were normal.

After 29 infusions of cerliponase alpha, at the age of 6 years and 10 months, we observed a stabilization of his condition under enzyme replacement therapy: no increase in the number of seizures, no further deterioration in visual contact or walking. Cone and flicker responses were normal. Absence of combined a-wave amplitude and increased combined b-wave peak time indicated that rods had abnormal responses.

After 80 infusions, at the age of 8 years and 9 months, absence of cone a-wave and cone b1-wave amplitudes indicated an abnormal functioning of cones. Absence of combined a-wave and b-wave response indicated that the scotopic system was no more functioning.

These results of our treated patient indicated a progressive abnormal retinal functioning, i.e., appearance of a progressive retinopathy. From normal at the age of 4 years and 5 months, retinas evolved to a retinopathy of *rod-dystrophy* type 2 years later at the age of 6 years 10 months, and to a *rod-cone dystrophy* 4 years later, at the age of 8 years 9 months. This secondary cone deficiency could be a consequence of the decrease in the trophic role of rods for the survival of cones [30, 31].

At third assessment, at the age of 8 years 9 months, it was not possible to quantify the child's visual acuity or evaluate his fundi aspects in order to compare them to his retinae which had an abnormal function because of disease progressions: hypotonia, severe myoclonia, irritability.

Binocular flash-VEPs when recorded before or after 29 infusions had abnormally high amplitudes, with an abnormal background noise. These abnormally high amplitudes are frequent in children with encephalopathy [32]. They probably reflected a high cortical activity more than the state of the macular or visual pathways.

In case of neurodegeneration with seizures, antiepileptic therapy could not control the seizures completely. After 80 infusions of enzyme replacement therapy, we observed a significant decrease in cortical activity: amplitude of binocular flash-VEPs being within normal with a normal background noise. It is not possible to interpret these results as a direct benefit of enzyme replacement therapy or as the expected worsening of cortical electrical activity with low voltage as described in CLN2 patients [33].

Infusions of cerliponase alpha brought a transitory stabilization of the neurological signs in our patient. The results of flash-ERG evolutions showed that rods were altered before cones.

In model animals, treated animals showed stabilization of neurological symptoms. However, the retinae of treated or untreated animals evolved in the same way as assessed by flash-ERGs. Photopic or mixed-ERG a-wave amplitudes were stable, whereas photopic and mixed-ERG b-wave amplitudes decreased drastically [34]. This meant that the rod and cone responses of animals situated in the first retina level functioned normally, whereas cone and rod bipolar cells situated in the second retina level were altered. Our results recorded on one treated child only differ from those. They have shown that rods were altered after 2 years of treatment corresponding to a *rod dystrophy*. Child's retinopathy continued to progress despite continued treatment. Four years after the first injection, child's retinopathy was a *rod-cone dystrophy*. However, it was not possible to compare these results to child's visual acuity or visual field.

Comparison between the evolution of the function with age in a human retina and canine retinae should be done with caution as the proportion of cones/rods and their locations differ [35].

Whiting et al. [34] observed preservation of the ganglion cells in treated animal models. They were identical to that of normal animals and seemed to have been preserved by treatment in animal models [16]. P-ERG recordings would have provided information on the functioning of the ganglion cells in our patient. However, given the child's age and physical condition, these recordings were not possible.

It would have been ideal to have combined OCT recordings with these flash-ERG and flash-VEP recordings in order to measure evolution of patient's retina thickness. This was not possible in our young patient due to his neurological condition. In addition, quantification of vision is frequently considered as secondary in those patients whose neurological or epilepsy problems are at the forefront.

Conclusion

Flash-ERGs recorded in our patient showed that the dysfunction of rods has preceded that of cones. The origin of this differentiated impairment over time is not clear. These results differ from those recorded in animal models. However, it is not possible to assert that the transitory preservation of cones is linked to the enzyme replacement therapy. We lack comparison with evolution of flash-ERGs recorded in untreated CLN2 subjects. Studying retinal function evolution without treatment or with a placebo treatment in these patients with a lethal disease is not ethically conceivable now that enzyme replacement therapy is available.

Declarations

Statement of human rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Statement on the welfare of animals This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflicts of interest None.

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