# Neuronal Ceroid Lipofuscinosis Type 2: A Case Series from Argentina

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**Abstract**

Neuronal ceroid lipofuscinosis type 2 (CLN2) disease is a rare autosomal recessive neurodegenerative disorder caused by mutations in the *CLN2/TPP1* gene, leading to a deficiency in tripeptidyl peptidase 1 activity. Enzyme replacement therapy with cerliponase alfa (recombinant human TPP1 [rhTPP1]; Brineura®) was approved in the United States and Europe for the treatment of CLN2 disease in 2017. We retrospectively report a cohort of 19 patients with CLN2 assisted in a specialized center in Argentina, including 8 newly diagnosed cases. Speech disorders and white matter changes/ventricular system enlargement were the most frequent clinical and imaging findings at CLN2 disease onset, respectively. Patients treated with cerliponase alfa presented a stable or improved course of the disease in this Latin American real world setting, as described in clinical trials.

**Keywords**

Neuronal ceroid lipofuscinosis, CLN, cerliponase alfa.

## Introduction

Neuronal ceroid lipofuscinoses (NCL) are a heterogeneous group of lysosomal storage disorders [1]. Among NCLs, neuronal ceroid lipofuscinosis type 2 (CLN2) is a rare autosomal recessive neurodegenerative, caused by mutations in the tripeptidyl peptidase 1 *(TPP1)/CLN2* gene, leading to a deficiency in TPP1 activity [1]. Low TPP1 induces lysosomal accumulation of autofluorescent material (ceroid), with increased neuronal apoptosis, neurodegeneration and a shortened lifespan [2]. Among the broad phenotypic spectrum of CLN2, the "classical" late infantile form or Jansky-Bielschowsky disease [3] and the variant juvenile or "protracted" form are highlighted [3–4].

CLN2 incidence ranges from 0.15 to 0.78 per 100,000 live births in Europe, with an estimated prevalence of 0.6-0.7 per million in Scandinavia [5]. Data from Latin America are scarce. In 2009, Kohan *et al* screened 118 subjects initially selected by clinical assessment of NCL-compatible signs and symptoms; laboratory tests confirmed the diagnoses of CLN2 in 9 patients [4]. After diagnosis, patients were examined for changes typical of NCL using brain magnetic resonance imaging (MRI), electroencephalogram, electroretinogram (ERG) and visual evoked potentials (VEPs) [4]. Histological evaluation of skin biopsies, enzymatic assays and mutation determinations were also detailed [4].

According to the DEMCHILD registry and Weill Cornell Medical College data set, the classical phenotype presents with rapid progression, which occurs in most CLN2 patients between the ages of 3 and 6 years, in a quick succession after the onset of first symptoms (regression of motor and language skills, development of myoclonus, decline in vision and deterioration of cognitive function) [6]. Affected children usually become highly dependent upon caregivers, with frequent respiratory infections, feeding disturbances, seizures and a fatal outcome [6]. In 2017, an intraventricular administrated enzyme replacement therapy (cerliponase alfa, recombinant human TPP1) has granted regulatory approval for treatment of CLN2 in the United States and Europe, marking the first disease-specific therapeutic strategies for CLNs [6]. Some Latin American countries have also granted access to this therapy.

### *Aims*

Main objectives of our study were: [1] to characterize newly diagnosed patients, including a subset of subjects treated with cerliponase alfa; [2] to compare the retrieved data with an historical cohort of CLN2 patients with a battery of clinical and complementary tests, when feasible.

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## Methods

### Data retrieval

Medical records of patients assisted at a reference center in Córdoba City (Argentina) were retrospectively reviewed, from October 2005 to November 2019 (cut-off date). The inclusion criterion was a confirmed diagnosis of CLN2 according to both the determination of TPP1 enzymatic activity and the molecular analysis of the *TPP1/CLN2* gene. Patients unwilling to undergo the proposed tests after written consent to assess the disease status were excluded.

The historical cohort included [a] Argentinean participants of a previous large case series from Latin America [4] and [b] any other patients who were assisted in the reference center who were diagnosed up to January 2011. All CLN2 confirmed patients diagnosed up to cut off date were considered as newly diagnosed.

Demographic data (date of birth, age at diagnosis), clinical data (presenting and follow-up symptoms, clinical and ophthalmological examination), biochemical and molecular data (age at the time of diagnostic tests and their results) were retrieved. The Hamburg Motor and Language (HML) score was calculated according to clinical symptoms, as part of usual clinical monitoring (Table 1) [7]. Data from laboratory, imaging and electrophysiological studies were also obtained. In patients under treatment with cerliponase alfa, corresponding data were included.

**Table 1.** The Hamburg Motor and Language scale.

| | Score | Functionality |
|---|---|---|
| Motor | 3 | Walks normally (grossly normal gait. No prominent ataxia, no pathologic falls) |
| | 2 | Independent gait (ability to walk without support for 10 steps). Will have obvious instability, intermittent falls and obvious clumsiness |
| | 1 | Only can crawl or requires external assistance to walk |
| | 0 | Immobile, mostly bedridden, can no longer walk or crawl |
| Language | 3 | Apparently normal language. Intelligible and grossly age-appropriate. No decline noted yet (individual maximum) |
| | 2 | Language has become recognizably abnormal. This score signifies a decline from the individual maximum reached by the child. |
| | 1 | Hardly understandable |
| | 0 | Unintelligible, no language or vocalizations |

(Adapted and modified from Wyrwich KW et al.) [7]

### Statistical analysis

The dataset was anonymized. The resulting cohort was divided according to two different criteria: [1] by differentiating the cases of surviving patients (historical cohort) from those with a newly CLN2 diagnosis, regardless of treatment; [2] by considering the clinical phenotype: classical (age of onset between 2 and 4 years, starting with tonic-clonic or partial seizures and delayed language development) *versus* atypical (later onset, generally starting with other manifestations including movement disorder and ataxia). All obtained data were tabulated in a Microsoft Excel® spreadsheet. A descriptive statistical analysis was performed: numerical variables were described according to central tendency and dispersion while categorical variables were described in terms of absolute and relative frequency. When feasible, a comparison was performed between data from the historical and newly diagnosed cohorts and from classical or atypical phenotype.

## Results

### Demographic data

Data from 19 patients were collected. Eight subjects were classified to be newly diagnosed and 11 were considered as part of the historical subgroup. Half of the newly diagnosed patients were receiving specific therapy.

Five subjects from the historical subgroup were died at cut-off date (four male and one female). Three deceased participants presented with clinical phenotype (including the female patient) and two had an atypical phenotype. Available demographic data from medical records was similar to the corresponding alive patients.

When divided according to the clinical phenotype, 13 of the 19 patients presented with the classical late infantile type, five subjects were defined as atypical phenotype (earlier or later symptom onset, or protracted course) and one patient could not be clearly defined due to missing data. Six of the eight newly diagnosed patients had a classical presentation.

Main demographic data for all subgroups are summarized in tables 2 and 3.

**Table 2.** Demographic data according to recent versus historical diagnosis.

| | Newly diagnosed | Historical cohort |
|---|---|---|
| N | 8 | 11 |
| Alive, n (%) | 8 (100%) | 6 (54.5%) |
| Male, n (%) | 6 (75%) | 8 (72.7%) |
| Age, years*, median (range) | 11.7 (4.9–17.8) | 19.63 (5.46–25.27)** |
| Patients receiving cerliponase alfa, n (%) | 4 (50%) | 0 (0%) |

* By cutoff date (November 28th 2019)
** Only alive patients at cutoff date were considered

### Clinical data

In the newly diagnosed subgroup, median age of clinical onset was 3.37 years (range: 3 to 7 years). In 25% of cases, an affected sibling was identified. Median age of clinical onset was similar in the historical cohort (median age: 3.3; range: 2.5 to 4.3).

**Table 3.** Demographic data according to clinical phenotype*.

| | Classical phenotype | Atypical presentation |
|---|---|---|
| N | 13 | 5 |
| Alive, n (%) | 11 (84.6%) | 2 (40%) |
| Male, n (%) | 9 (62.9%) | 4 (80%) |
| Age, years**, median (range) | 13.4 (4.9-25.3) | 9.15 (4.9-13.4)*** |
| Patients receiving cerliponase alfa, n (%) | 2 (15.3%) | 2 (40 %) |

* Data available from 18 patients
** By cutoff date (November 28th 2019)
*** Only alive patients at cutoff date were considered
Small sample sizes preclude a formal statistical comparison.

Considering the entire cohort, median time span from the initial symptoms to the diagnosis was 5.5 years (range: 1.2-12.7). No differences were demonstrated among both subgroups (data not shown). In all patients, reduced activity of tripeptidyl peptidase in leukocytes was confirmed. Among subjects with mutational analysis, C.827A>T and c.622C>T were the most frequent mutations, even though no definite predominance was found.

The most prevalent presenting symptom among newly diagnosed patients was speech disorder, mainly delay in speech development. By contrast, seizures were the main presenting symptom in the historical cohort (Figure 1). Seizure pattern was highly variable, without a predominant type.

Main symptoms observed during follow-up in the newly diagnosed CLN2 patients are described in Figure 2.

## *Central nervous system imaging*

Both in the historical cohort and in newly diagnosed patients, the first brain MRI was performed at a median age of 4.8 years (respective ranges: 2.5-11 and 2.75-24 years). The most frequent initial structural alterations are summarized in Figure 3. No significant correlations were found between the frequency of such anomalies (corpus callosum alterations, mainly thinning; *gyri* and *sulci* alterations, mainly widening; cerebellar atrophy; T1-weighted hyperintensities in periventricular white matter; ventricular system dilation) and the initial symptom or age at diagnoses.

## *Visual function*

Respectively, 87.5% and 81.8% of newly diagnosed patients and participants from the historical cohort presented self-reported or caregiver-reported functional or structural anomalies in the ophthalmological examination (ophthalmoscopy, ERG, VEPs) performed at diagnosis. Data related to ophthalmological follow-up were scarce.

## *Cerliponase alfa treated patients*

Four of the eight newly diagnosed patients were under enzyme replacement treatment. Two of them were diagnosed as classical phenotype and the other two were defined as atypical.

Age at first symptom in subgroup of treated patients was four years (range: 3.25 to 7). In addition, diagnosis was performed at a median age of 7.2 years (range: 2 to 10.4), with similar median and ranges both for typical (n = 2) and atypical (n = 2) presentations.

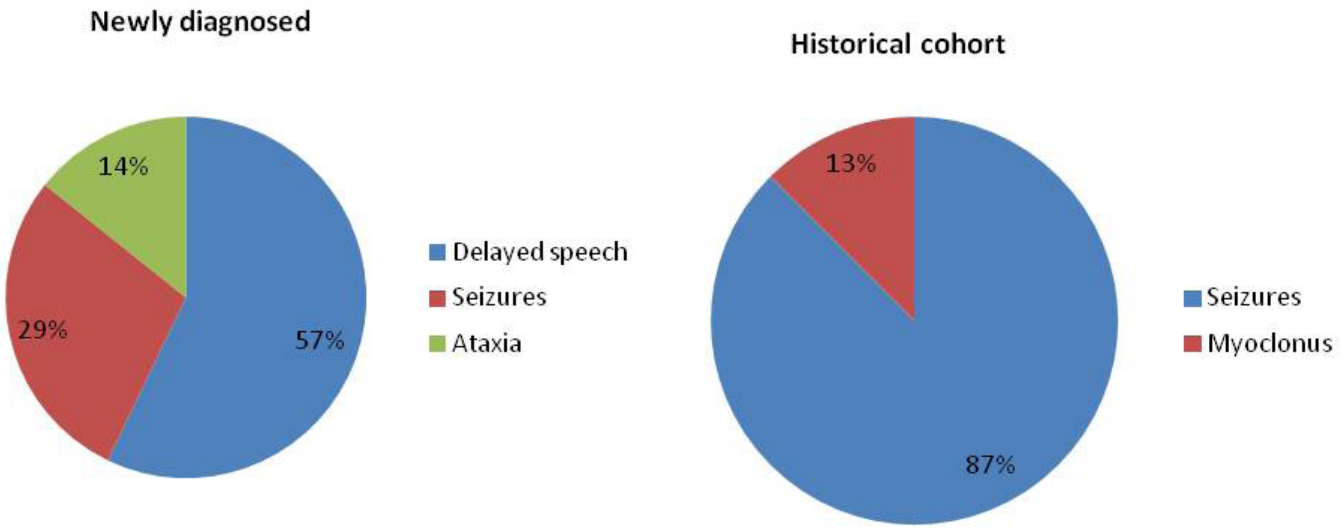


**Figure 1.** Most frequent initial symptoms among newly diagnosed and historical CLN2 patients.
Definitions for historical and newly diagnosed cases are detailed in "Methods" section.

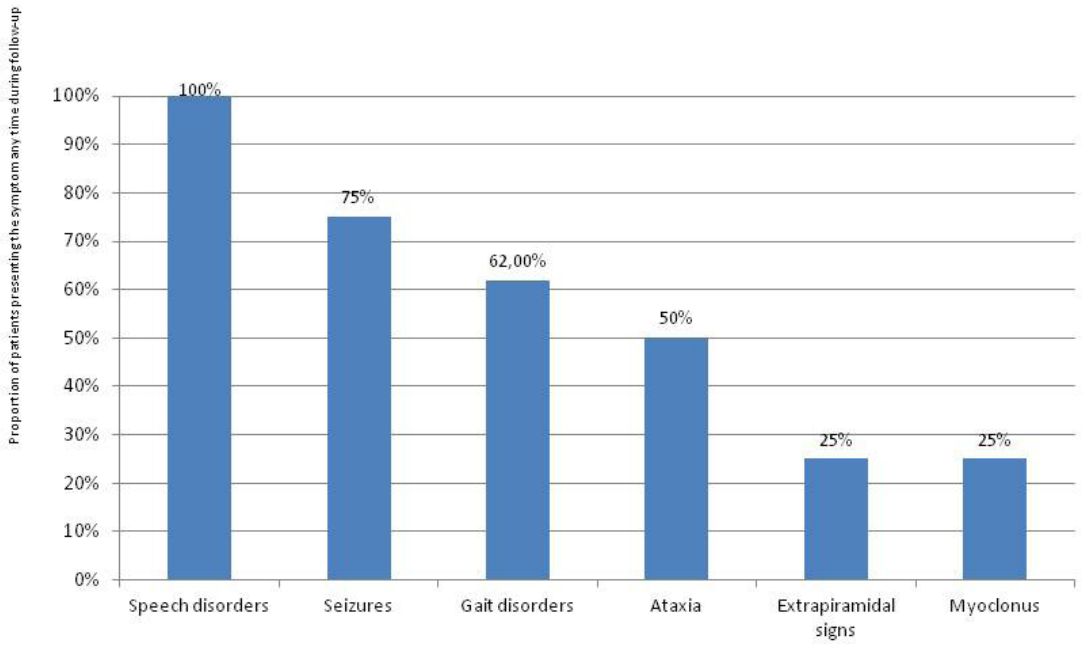


**Figure 2.** Symptoms described during follow-up in newly diagnosed patients (n = 8).

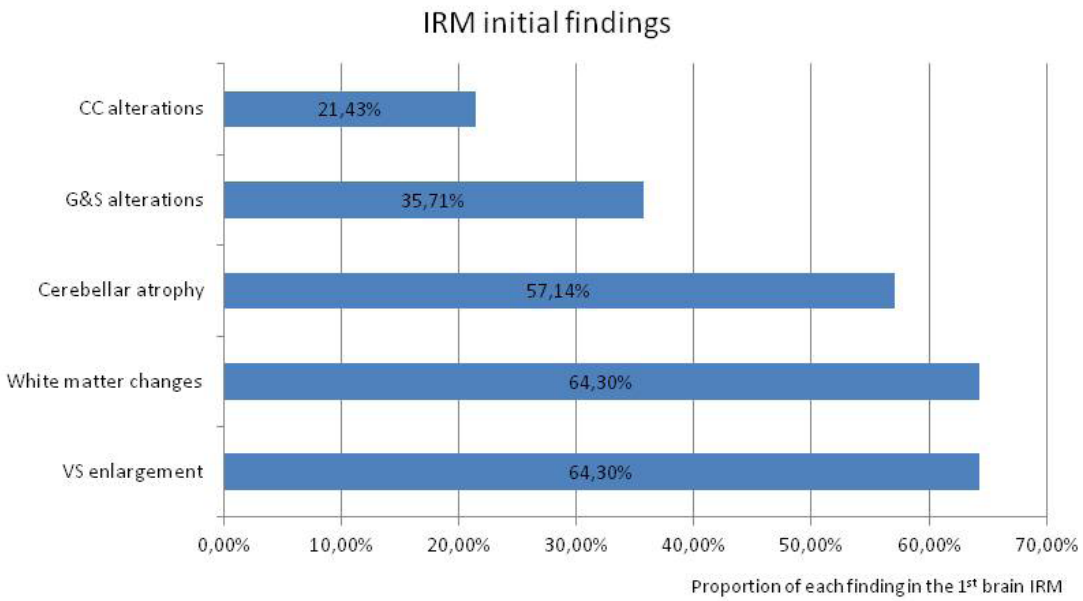


**Figure 3.** First brain IRM findings among CLN2 patients (n = 15).
CC: Corpus callosum; G&S: *gyri* and *sulci*; VS: ventricular system.

In this subgroup of treated patients, delayed speech and/or language impairment was the initial symptom in two cases, while ataxia and seizures were the respective initial clinical manifestations in each remaining subject.

At the time of the cutoff date, patients had received treatment during 2.3 years (range 3.25 to 7). No treatment-related or intracerebroventricular device-related complications were reported during follow-up. No clinical deterioration based on the Hamburg scale was recognized in two patients (one with classical phenotype and one with atypical initial presentation). A subject with classical phenotype and a null basal HML score had no clinical changes. In the remaining patient, who presented with an atypical phenotype, improvement of both motor and language function was evident during cerliponase alfa treatment (Figure 4). No difference was identified in MRI imaging before and after cerliponase alfa treatment. The small sample precludes a formal statistical comparison with other subgroups.

For comparative purposes, best available calculated HML scores based on medical records of patients of the historical cohort are described in Figure 5.

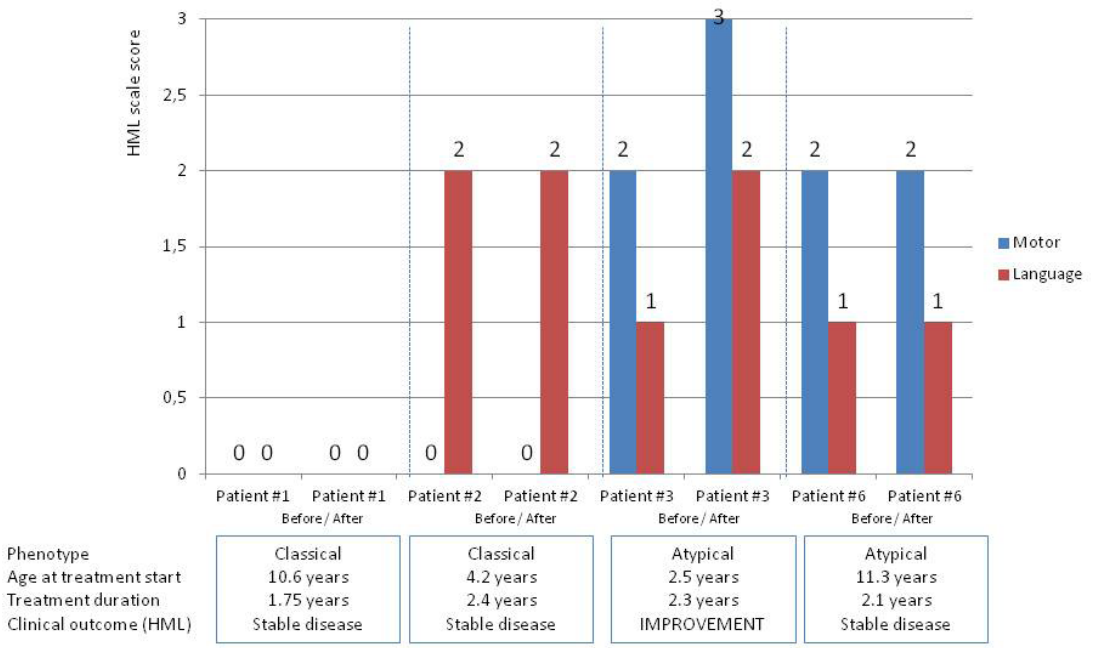


**Figure 4.** HML scale scores before treatment starting and at cutoff date among 4 cerliponase alfa treated patients. Boxes describe clinical phenotype, treatment characteristics (age at starting, therapy duration) and outcomes.
Patiens data have been anonymizes. HML: Hamburg Motir and Language Scale.

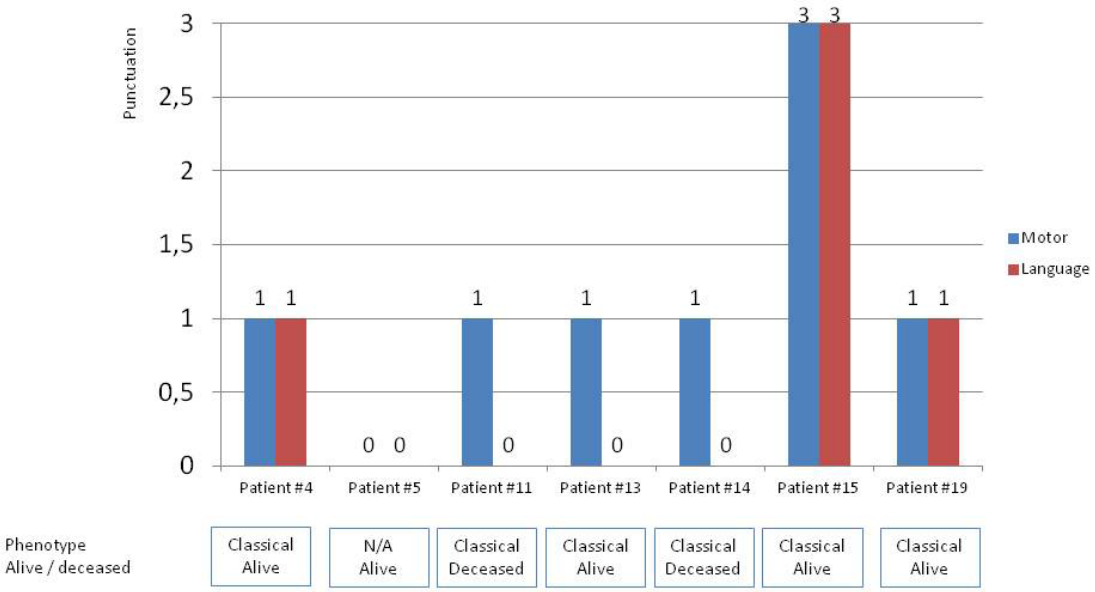


**Figure 5.** Best reported Hamburg Motor and Language scores in the historical cohort.
Patients data have been anonymized. Only available data are shown. Definition for historical cohort is detailed in "Methods" section.

## Discussion

In this comparison between a historical cohort of CLN2 patients and a group of newly diagnosed subjects, cerliponase alfa was associated with a stable or improved disease course.

CLN2 disease is a rare, severe and progressive neurodegenerative disorder, with early mortality [8]. Diagnosis should be carried out through both genetic testing for mutations in *CLN2/TPP1* and enzyme activity testing of TPP1 protein [6]. Main clinical and functional characteristics of CLN2 patients have been described elsewhere [3,9]; however, case series data in the era of enzyme replacement therapy are scarce in Latin America.

According to current literature, diagnostic delays are frequent in patients without a history of CLN2 disease among their relatives, with a reported delay between the onset of symptoms and a definitive diagnosis that may span at least 3 years [6]. In our

cohort, this time lapse was longer, with a median of 5.5 years. Limited awareness of CLN2 disease, atypical clinical presentation and misdiagnoses with non-specific language disorders or epileptic syndromes may be considered as potential reasons for this delay [6]. Given the broad range of language skills in early childhood in the general population, language alterations may be overlooked as a key initial symptom of CLN2 disease. It is worth noting that speech disorders were the most frequent initial symptom among our newly diagnosed patients. Seizures are frequently reported at an early disease stage in CLN2 literature, but language delay or deficits are becoming increasingly noted as the first symptom [6]. We hypothesize that this difference is due to a recent higher pediatrician aware of speech delay or alterations as a forerunner of CLN2 disease, leading to a more intensive screening in the newly diagnosed subgroup.

MRI is frequently indicated in the first stages of assessing any patient with seizures. In CLN2 disease patients, detection of often missed and subtle signs of atrophy may increase the likelihood of early diagnosis [6]. In our cohort, a first MRI was performed at a median age of 4.8 years, probably due to atypical presentation, and ventricular system enlargement, white matter changes and cerebellar atrophy were reported in more than a half of these subjects. In a previous Italian study of 14 patients with CLN2 disease, a first brain MRI was performed earlier (median age: 3.8 years) and cerebellar atrophy was identified in 100% of cases, while periventricular white matter changes were found in 79% of patients [10–12]. These differences among Latin American subjects clinical and imaging phenotypes and other cohorts deserve further research. CLN2 disease is the most common form of lipofuscinosis in South America; a later onset of symptoms and slower neurodegenerative progression, when compared with the classical form, may account for more than 50% of the cases described in our region, unlike other areas of the world [15]. Wide genetic mix and the finding of different mutations and deletions observed in South America may explain this difference [15].

Cerliponase alfa (recombinant human TPP1) has been approved as an enzyme replacement therapy for CLN2 disease patients in the United States and Europe, among other. In a pivotal study including 23 patients with CLN2 disease with a median age of 58 months at enrollment, intraventricular infusion of cerliponase alfa resulted in a significant reduction in the rate of decline of motor and language functions, in comparison with a natural history population [11]. Adverse events described during a follow up of at least 1.8 years included seizures, pyrexia, vomiting and failure of the intraventricular device, including infections leading to device replacement [13]. It should be noted that, according to previous research, [12,14] a better awareness of CLN2 disease may lead to an earlier diagnosis and a prompt initiation of specific enzyme replacement treatment.

Even though our cohort included only four treated patients, it should be emphasized that this subgroup experienced a stable or improved disease course, as described in the pivotal trial. It is highlighted that no drug related events adverse were described in our population and no patient needed a device replacement during a median follow-up of 2.3 years. Even though the initial HML score was zero in one of our patients, no further clinical deterioration was perceived and CLN2 disease remained stable with cerliponase alfa treatment.

Several limitations of our study include the small sample size, its retrospective design and the exclusive inclusion of live patients in the historical cohort, which may overestimate median age of the subgroup. However, our research confirms the effectiveness and safety profile of cerliponase alfa treated CLN2 patients in Argentina, both classical and atypical presentations.

## Conclusion

CLN2 is characterized by regression of motor and language skills, myoclonus, visual impairment and deterioration of cognitive function. In our analysis of an historical cohort of CLN2 patients and a set of newly diagnosed subjects, cerliponase alfa was associated with a stable or improved disease course, as inferred from Hamburg scale results.

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### Declaration of Conflicting Interests

The study protocol was approved by institutional ethics committee at participating institution. The study was done in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

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