“Real world effectiveness of cerliponase alfa in classical and atypical patients. A case series”

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A R T I C L E   I N F O

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A B S T R A C T

Introduction: Late infantile neuronal ceroid lipofuscinosis is an autosomal recessive disease caused by mutations in the CLN2/TPP1 gene, with secondary enzyme deficiency. In classical phenotypes, initial symptoms include seizures and delayed language development between 2 and 4 years of age. This article describes the presentation of CLN2 disease in a cohort of Colombian patients, as well as the impact of treatment on the course and progression of the disease.

Methods: Case series report of 8 patients with a confirmed diagnosis of neuronal ceroid lipofuscinosis treated with cerliponase alfa who remained on clinical and paraclinical follow-up for up to 24 months before and after treatment.

Results: An atypical phenotype, associated with initial symptoms and late diagnosis, was present in 5/8 patients. The most frequent symptoms were seizures and developmental delay, with age of onset at 24 months (classical phenotype) and 48 months (atypical phenotype). A novel mutation (c.1438G > A) was found in two siblings. All of the patients received cerliponase alfa, and there were no serious adverse events. No decline in the clinical status greater than 2 points on Hamburg, Weill Cornell and CNL2 clinical assessment scale was observed during follow-up after treatment initiation.

Conclusion: This is the first case series reported for neuronal ceroid lipofuscinosis patients in Colombia. In contrast with other reports, the majority of cases reported here displayed an atypical phenotype. Our study highlights the importance of early diagnosis and timely initiation of therapy, which is a feasible therapy, well tolerated by patients and accepted by caregivers in this country, generating a positive impact in the quality of life of CLN2 patients and on disease outcome.

1. Introduction

Neuronal ceroid lipofuscinoses (NCLs) are lysosomal storage diseases that, collectively, represent the most common group of infantile encephalopathies [1]. Late infantile neuronal ceroid lipofuscinosis type 2 (CLN2) (OMIM#204500) [2], is an autosomal recessive disease caused by mutations in the CLN2/TPP1 gene located on chromosome 11p15.4 which encodes the tripeptidyl-peptidase 1 (TPP1) enzyme [3]. To date, a total of 131 independent mutations of the CLN2/TPP1 gene have been reported, with a phenotypic description in 65%, and 66% of these mutations being familiar genotypes [4]. The most common mutations are c.509-1G > C and c.622C > T [p.Arg208X] which, taken together, account for 60% of the mutational spectrum of the disease, with genotype depending on the geographic region [4].

Worldwide, the reported incidence is 0.1–7/100,000 and varies by geographic region [5,6]. In Latin America in 2015, after a 12-year follow-up, the integrated program in Argentina reported a total of 22 patients from different Latin American countries [7]. To date, there are no reports on the incidence of this disease in Colombia.

The classical phenotype of the disease manifests in a previously healthy child. The initial symptoms vary: tonic-clonic or partial seizures (70% of cases) and delayed language development (57% of cases) with...
age of onset between 2 and 4 years [8,9]. Progressive motor and cognitive decline occur later, leading to the loss of voluntary movement, altered swallowing and speech (6 years), visual loss due to retinal atrophy (between 7 and 10 years) and death in mid adolescence (10–15 years) [8]. There are several variants of the classical phenotype, classified as atypical, which are characterized by late onset with variable clinical manifestations [10,11].

Diagnosis is confirmed by the presence of compatible homozygote or compound heterozygote mutations of the CLN2/TPP1 gene and TPP1 enzyme deficiency or absence [11]. Electroencephalographic (EEG) evidence of early photoparoxysmal response may be observed, starting at low frequencies (1–2 Hz), with irregular activity, attenuated background activity and epileptiform abnormalities in the occipital region [11]. Brain nuclear magnetic resonance imaging (MRI) may show early evidence of progressive cerebral and cerebellar atrophy which is a key diagnostic finding suggestive of CLN2. Other findings include: reduced grey matter volume and hyperintense periventricular white matter [12]. As part of the ophthalmological assessment, optic coherence tomography may show evidence of retinal degeneration [13] and accumulation of hyperreflective material [14], abnormal visual evoked potentials and diminished electroretinographic amplitudes [14].

There are multiple disease-specific scales that measure progression of the disease and allow assessment of the degree of disability based on different characteristics such as motor and visual function, gait and language (Table 1).

Patients with CLN2 disease require multidisciplinary management in order to address symptoms, such as distress, maintain motor function and improve/maintain quality of life depending on the clinical status at the time of diagnosis.

Recombinant human TPP1 (cerliponase alfa) is the only available disease-modifying therapy and has been shown to slow the progression of decline in the motor-language domains of the CLN2 Rating Scale, when compared to patients receiving symptomatic management alone in the study of the natural history of the disease [18]. This article describes the presentation of CLN2 in a cohort of Colombian patients, as well as the impact of the treatment on the course and progression of the disease.

2. Materials and methods

We present a case series report of 8 patients from three regions of Colombia (Bogotá, Villavicencio and Armenia); patients were seen in four centers (Fundación Hospital Pediátrico la Misericordia, Hospital Central de la Policía Nacional, Hospital Universitario Clínica San Rafael in Bogotá; and Neuroconexión en Armenia) and followed by a multidisciplinary team for a time period of 24 months. Patients had a diagnosis of CLN2, of classical or atypical phenotypical expression, confirmed by biochemical and enzymatic testing and genotyping of the CLN2/TPP1 gene. Atypical patients were those with enzymatic and/or molecular diagnosis of CLN2 who exhibited a protracted or milder clinical course, with variation in age at onset, rate of progression and clinical manifestation, as compared to the classical late-infantile phenotype [19]. Enzyme replacement therapy was offered in accordance to local regulatory guidelines for patients with confirmed diagnosis of CLN2, aged 3–15 years with motor-language Hamburg score within 3–6 [20].

Data was collected retrospective based on clinical charts with a prospective follow-up after treatment. Patient variables included in accordance with the protocols for clinical record reviews at each of the centers: a) Descriptive variables (age, gender, affected relatives, consanguinity and perinatal history); b) Variables pertaining to the onset of the disease (age of onset, initial symptoms, symptoms on first visit, initial pharmacological management, initial paraclinical testing and TPP1 sequencing); c) Follow-up variables (additional symptoms over the course of the disease, scores on the CLN2 Clinical Rating Scale, Weill Cornell scale, Hamburg scale and Hamburg motor and language scale); d) Variables pertaining to enzyme replacement therapy (starting age, dose, frequency, time on therapy, adherence percentage), and variables related to the course of the disease after treatment (scores on the CLN2 Clinical Rating Scale, Weill Cornell scale, Hamburg motor and language scales).

An Excel (version 16.35) database was built including the variables described above, and statistical analysis was performed using the SPSS software package (version 26). Descriptive and inferential statistics were applied. Alfa error of 0.05, beta error of 80%, and a 95% confidence interval (CI) were established, with a statistically significant p value <0.05. Non-parametric tests (Wilcoxon) were performed given the non-normal sample distribution. Verification of 100% of the data was carried out for quality control in order to avoid information bias. Data anonymization was applied in order to ensure confidentiality, considering the sensitive nature of the information.

This article is in compliance with the Nuremberg Code of 1947 [21], the Universal Declaration of Human Rights of 1948 [22], Resolution 8430 of 1993 [23] and the Declaration of Helsinki [22]. Ethics Committee of Fundación Hospital Pediátrico la Misericordia (HOMI) (No. CEI-259-20) granted authorization for the study and signed informed consents for the use of the information was obtained from the guardians of each of the patients.

3. Results

This case series report considered 8 patients with a phenotypical diagnosis of CLN2 with enzymatic, biochemical and TPP1 genotyping (Annex 1). An atypical phenotype was found in 5/8 of the patients. There were two pairs of siblings in the cohort of patients, both families having the atypical phenotype: One of the patients received diagnostic confirmation while still asymptomatic because of the history of an older sibling, who was previously identified.

At the time of the first visit, 7/8 of the patients presented with seizures, 6/8 with language abnormalities, 4/8 with developmental regression, and 3/8 with ataxia and movement disorders. The initial symptoms by subgroups are shown in Table 2, the most predominant being seizures in both the classical (3/3 and 5/5) and the atypical phenotypes (4/5). During clinical follow-up up to the time of diagnosis, and before enzyme replacement therapy, disease progression continued as illustrated in the table.

Medians and respective inter-quartile ranges for age of symptom onset, age at first visit, age of developmental regression, time from diagnosis to initiation of therapy, age at treatment initiation, and time on therapy are described in Table 3; 6/8 of the patients had an affected relative and none of the patients had a history of consanguinity.

During follow-up after the first visit and before the start of enzyme replacement therapy, 3/8 patients developed ataxia, 3/8 developed movement disorders and 2/8 regression in their language development. In the subgroup analysis, among patients with the classical phenotype 2/3 patients (developed ataxia, 2/3 patients developed movement

| Tool | Measurement | Author and validation |
|------|-------------|-----------------------|
| Hamburg Scale | Motor, language and visual assessment | Steinfeld, [15] |
| | Total score 9 (3 for each category) | |
| | Separate item for grand mal seizures, not taken into account in the total score | |
| Weill Cornell Scale | Swallowing, gait, motor function and language assessment | Worgall, [16] |
| | Total score 12 (3 for each category) | |
| CNL2 clinical assessment scale | Motor, language assessment, and combination of the two | Wyrvich, [17] |
| | The combined total score is 6. | |
disorders, 2/3 patients developed developmental regression, and 3/3 patients developed ophthalmological alterations. On the other hand, among patients with atypical phenotypes, 1/5 patient developed ataxia, 1/5 patient developed movement disorder, and 1/5 patient developed ophthalmological alterations, while one patient remained asymptomatic; the other findings were unchanged (Table 2). The EEG, MRI and other additional tests of patients with the classical and the atypical phenotypes are described in Table 2. Levels of tripeptidyl-peptidase 1 enzyme activity were undetectable or below the normal range in 8/8 of the patients in both groups. Table 4 shows the data for CLN2/TPP1 gene sequencing.

As part of the treatment received before starting on enzyme replacement therapy, 6/8 of the patients were receiving valproic acid, 2/8 levetiracetam, 3/8 clobazam, 1/8 oxcarbazepine and 1/8 lamotrigine (Table 5).

### Table 2
Qualitative clinical characteristics of the patients before initiating enzyme replacement therapy.

| Symptoms                          | Classical (n = 3) | Atypical (n = 5) | Symptoms prior enzyme replacement therapy |
|-----------------------------------|------------------|-----------------|------------------------------------------|
| Symptom                           |                  |                 | Classical (n = 3) | Atypical (n = 5) |
| Asymptomatic                      | 0                | 1               | 0                         | 1               |
| Language abnormalities            | 3                | 3               | 3                         | 3               |
| Movement disorders                | 1                | 1               | 3                         | 2               |
| Ophthalmological alterations      | 0                | 0               | 3                         | 1               |
| Ataxia                            | 1                | 2               | 3                         | 3               |
| Seizures                          | 3                | 4               | 3                         | 4               |
| Developmental regression          | 1                | 3               | 3                         | 3               |
| Additional tests                  |                  |                 |                           |                 |
| EEG (abnormal)                    | 3                | 3               |                           |                 |
| Electroretinogram (abnormal)      | 3                | 0               |                           |                 |
| Visual evoked potentials (abnormal)| 0               | 1               |                           |                 |
| Photoparoxysmal response (abnormal)| 0              | 1               |                           |                 |
| Brain MRI (abnormal)              | 3                | 2               |                           |                 |
| Optic tomography (abnormal)       | 2                | 0               |                           |                 |

### Table 3
Quantitative clinical characteristics.

| Case series | Median | Range (Q1-Q3) | Median | Range (Q1-Q3) | Median | Range (Q1-Q3) |
|-------------|--------|---------------|--------|---------------|--------|---------------|
| Age of onset of symptoms (months)  | 36     | (30–48)       | 24     | [24–36]       | 48     | (36–48)       |
| Age at the time of the first visit (months) | 48     | (30–66)       | 30     | [30–33]       | 60     | (60–84)       |
| Age of developmental regression (years) | 3      | (2–10)        | 3      | (3–3)         | 3.5    | (2–10)        |
| Time from diagnosis to therapy initiation (months) | 11     | (3–13)        | 4      | (3–11)        | 13     | (5–13)        |
| Age at treatment initiation (months)  | 72     | (48–192)      | 52     | (48–62)       | 139    | (72–192)      |
| Time on therapy (months)  | 9      | (7–24)        | 16     | (15–24)       | 8      | (7–9)         |
| Time from onset of symptoms until diagnosis (months) | 12     | (11–12)       | 6      | (5–6)         | 24     | (21–27)       |

### Table 4
TPP1 sequencing.

| Phenotype  | Allele          | Location | Nucleotide change | Aminoacid change | Clinical significance | Contig position (GRCh38p7) | Reference     |
|------------|-----------------|----------|-------------------|------------------|-----------------------|----------------------------|---------------|
| Atypical   | Compound        | Exon 12  | c.1438G > A /     | p.Val480Met /    | NA / Probably pathogenic | 6614979C > T /            | NA / Caillaud et al., 1999 |
|            | heterozygous    | Intron 8 | c.1076-2A > T    | NA               | NA / Probably pathogenic | 6,616,076 T > C           | NA / Caillaud et al., 1999 |
| Atypical   | Compound        | Exon 12  | c.1438G > A /     | p.Val480Met /    | NA / Probably pathogenic | 6614979C > T /            | NA / Caillaud et al., 1999 |
|            | heterozygous    | Intron 8 | c.1076-2A > G    | NA               | NA / Probably pathogenic | 6,616,076 T > C           | NA / Caillaud et al., 1999 |
| Atypical   | Compound        | Exon 6   | c.622C > T /     | p.Art2808 /     | Pathogenic / Conflicting | 6,617,040 G > A /         | Slet et al., 1999 / Noher de Halac et al., 2005 |
|            | heterozygous    | Intron 7 | c.887-10A > G    | Varia            | Pathogenic / Conflicting | 6,615,513 T > C          | Slet et al., 1999 / Noher de Halac et al., 2005 |
| Atypical   | Compound        | Exon 6   | c.622C > T /     | p.Art2808 /     | Pathogenic / Conflicting | 6,617,040 G > A /         | Slet et al., 1999 / Noher de Halac et al., 2005 |
|            | heterozygous    | Intron 7 | c.887-10A > G    | Varia            | Pathogenic / Conflicting | 6,615,513 T > C          | Slet et al., 1999 / Noher de Halac et al., 2005 |
| Atypical   | Compound        | Exon 8   | c.1076-2A > A    | NA / Varia       | Probably pathogenic /   | 6,616,076 T > C /         | Caillaud et al., 1999 / 2005 |
|            | heterozygous    | Intron 7 | c.887-10A > G    | NA / Varia       | Conflicting             | 6,615,513 T > C           | Noher de Halac et al., 2005 |
| Classical  | Compound        | Exon 8   | c.1076-2A > T    | NA / p.          | Probably pathogenic /   | 6,616,076 T > C /         | Caillaud et al., 1999 |
|            | heterozygous    | Intron 7 | c.887-10A > G    | NA / p.          | Pathogenic              | 6,615,513 T > C           | Noher de Halac et al., 2005 |
| Classical  | Compound        | Exon 5   | c.471C > A /     | p.Tyr157* / NA  | Probably pathogenic /   | 6,617,338C > A            | Sheth et al. 2018 / Caillaud et al., 2012 |
|            | heterozygous    | Intron 8 | c.1076-2A > G    | NA / p.          | Probably pathogenic /   | 6,610,076 T > C           | Sheth et al. 2018 / Caillaud et al., 2012 |
| Classical  | Compound        | Exon 8   | c.1076-2A > T    | NA / p.          | Probably pathogenic /   | 6,616,076 T > C           | Caillaud et al., 1999 / 2005 |
|            | heterozygous    | Intron 6 | C.616C > T       | arg206Cys        | Pathogenic              | 6,616,046 G > A           | Caillaud et al., 1999 / 2005 |

### Table 5
Treatment prior and post enzyme replacement therapy.

| Initial treatment | Additional treatment post enzyme replacement therapy |
|-------------------|------------------------------------------------------|
| Classical (n = 3) | Atypical (n = 5) | Classical (n = 3) | Atypical (n = 5) |
| Valproic acid     | 3 | 3 | 3 | 1 |
| Clobazam          | 3 | 0 | 3 | 1 |
| Lamotrigine       | 0 | 1 | 0 | 0 |
| Levetiracetam     | 1 | 1 | 1 | 2 |
| Oxcarbazepine     | 1 | 0 | 1 | 0 |
Table 6
Functional scales before and after treatment.

| Functional scales | Case series | Classical phenotype | Atypical phenotype |
|-------------------|-------------|---------------------|-------------------|
|                   | Median (Range) | Median (Range) | Median (Range) |
| Before treatment  | CLN2 Clinical scale | 4 (3-4) | 2 (2-3) | 4 (4-5) |
|                   | Rating Scale | 8 (6-10) | 6 (6-7) | 8 (5-10) |
|                   | Well-Cornell scale | 9 (7-10) | 6 (5-7) | 10 (9-11) |
|                   | Hamburg scale | 4 (3-4) | 3 (3-4) | 4 (4-5) |
|                   | Weill-Cornell scale | 7 (5-10) | 5 (5) | 8 (5-10) |
|                   | Hamburg scale | 9 (7-10) | 10 (9-11) | 7 (6-7) |
|                   | Hamburg ML scale | 4 (3-4) | 3 (3-4) | 4 (4-5) |
| After treatment   | CLN2 Clinical scale | 4 (2-4) | 2 (2) | 4 (4-5) |
|                   | Rating Scale | 7 (5-10) | 5 (5) | 8 (5-10) |
|                   | Well-Cornell scale | 9 (7-10) | 10 (9-11) | 7 (6-7) |
|                   | Hamburg scale | 4 (3-4) | 3 (3-4) | 4 (4-5) |
|                   | Hamburg ML scale | 4 (3-4) | 3 (3-4) | 4 (4-5) |

All of the patients received intracerebroventricular cerliponase alfa 300 mg every 15 days as enzyme replacement therapy, including one asymptomatic patient with enzymatic activity below the detection range. Median time from diagnosis to initiation of therapy was 11 months (IQR 3-13). Median time from treatment initiation until the study cut-off point (June 2020) was 9 months (IQR 7-24). Subgroup analysis revealed that, compared to patients with the classical phenotype, patients with atypical phenotypes showed older median age of onset (139 months vs. 52 months), longer median time from onset of symptoms until diagnosis (24 months vs. 6 months), longer median time from confirmation of diagnosis to initiation of treatment (13 months vs. 4 months), and shorter time on therapy (8 months vs. 16 months) (Table 3).

The analysis after initiation of enzyme replacement therapy with cerliponase alfa showed evidence of reduction in the use of valproic acid in the atypical phenotype patients (3/5 vs. 1/5), while there was increased use of levetiracetam (1/5 vs. 2/5) and clobazam (0/5 vs. 1/5). Medication use remained constant in the classical phenotype (Table 5).

Functional scores were analyzed before and after treatment initiation, as shown in Table 6. The statistical analysis did not show evidence of statistically significant differences between pre and post-treatment scores (p > 0.005).

The median time on enzyme replacement therapy was 9 months. Fig. 1 shows median scores on the CLN2 and Hamburg scales before and after treatment. Fig. 2 shows individual patient scores on the CLN2 scale during follow-up before and after treatment initiation.

Data from both atypical and classical phenotypes. Boxes show median values (middle lines) with a 25th and 75th percentiles; whiskers show values within two-thirds of the IQR. CLN2 = Late-infantile neuronal ceroid lipofuscinosis type 2.

Longitudinal follow up (in weeks) of CLN2 scale on individual patients according to their clinical phenotype.

During follow-up, there was no evidence of progression, defined as 2-point decline in the CLN2 scale (Fig. 3).

Data from both atypical and classical phenotypes. Bars show changes from baseline (prior to treatment) in CLN2 score during follow up. No progression was evident (defined as > − 2 in CLN2 scale).

4. Discussion

To date, multiple genetic variants responsible for CLN2 disease have been described, with variable population allele frequencies. On CLN2/TPP1 gene sequencing, the most frequent allele (6 of 16) in our case series was c.1076-2A > T (rs1424116749), an intronic variant in which the base replacement in region 3’ changes the CLN2/TPP1 gene splicing acceptor [25]. It has been described as a potentially pathogenic variant and, moreover, it was present in all patients with the classical phenotype, accompanied by a second allele with a pathogenic mutation due to an amino acid (c.616C > T) or a stop codon (c.471C > A) replacement (Table 3). None of these mutations correspond to the most frequent genotypes in patients with CLN2 in the world [4], demonstrating the genetic heterogeneity and population variation of genetic diseases such as CLN2. This also reinforces the difference between the mutational profile in the Latin American population and profiles reported in other regions of the world.

In patients with the atypical phenotype, the most frequent allele variant (3 of 10) was c.887-T10A > G (seen on the two siblings in the study), an intronic variant described for the first time in Portugal [26] and reported as the most frequent variant on the largest cohort described [27]. The authors postulate that the protein with this mutation could have residual activity. A nonsense mutation was also found in two patients (c.622C > T) while the c.1076-2A > G mutation described previously (altered splicing) was found in a third patient. The two remaining patients had one allele with a splicing alteration and another allele with a mutation not previously described in the literature (c.1438G > A), the clinical meaning of which has not been described. The assumption could be that, given its association with an atypical phenotype, it might have a partial functionality in the resulting
transcript. Genomic variations in atypical phenotypes support the hypothesis of a combination of intronic and nonsense/missense mutations associated with residual enzyme activity, allowing for a phenotype with a milder presentation, as previously reported [28].

In our patients, the classical phenotypical presentation is consistent with the evidence described in the literature [14, 29, 30], with a median age of onset of 24 months at which 3/3 showed seizure activity and regression in terms of language development. In the atypical phenotype, the median age of presentation was 48 months, with a broader age range (36–120 months) and seizures as the predominant initial symptom (4/5), followed by language alterations and developmental regression (3/5). A later course of the disease and a less severe phenotype is seen when the two subgroups are compared, which is consistent with the descriptions of atypical phenotypes [7, 10]. The median interval from the onset of symptoms to the first visit was 3 years longer in the atypical phenotype patients, while time to diagnosis was 1.5 years longer, as well as time to initiation of enzyme replacement therapy (4 months in the classical phenotype vs. 13 months in the atypical phenotype). This may be explained by clinical variations and the later onset of symptoms in the atypical phenotype, which pose a diagnostic challenge, as evidenced in our study. Therefore, it is important to consider the possibility of CLN2 disease in patients with seizures and altered language development or

Fig. 2. Individual follow-up results for the case series according to clinical phenotype.

Fig. 3. Changes from baseline (prior to treatment) on the CLN2 scale during follow-up.
with CLN2 in Colombia. Evidence was found of a novel mutation in two patients with different allele frequency variants than those reported in the world literature. Evidence was also found of a higher number of patients with the atypical phenotype as compared to the classical phenotype in this case series, in contrast with international reports. However, the clinical characteristics identified in the two groups are consistent with those described previously in the literature. There is a prolonged latency between the onset of symptoms and diagnosis, particularly in the atypical phenotype, reflecting the detection challenge in patients of this region. In the CLN2 disease patients included in this case series, treatment with enzyme replacement therapy resulted so far, in no progression of motor and language decline, as determined using scales for clinical reassessment during follow-up. This study is among the first to report real-world evidence of effectiveness of the medication, with similar results to those reported in the world literature and a similar response to the use of cerliponase alfa in patients with both the classical and the atypical phenotypes. Additionally, our study highlights the importance of early diagnosis and timely initiation of therapy, which is a feasible therapy, well tolerated by patients and accepted by caregivers in this country, generating a positive impact in the quality of life of CLN2 patients and on disease outcome.

**Ethics committee**

This study was approved by the Ethics Committee of Fundación Hospital Pediátrico la Misericordia (HOMI), Bogotá, Colombia (No. CEI-259-20). A signed informed consent for publication of the clinical cases, laboratory results, paraclinical testing, photographs and videos was obtained from the children's parents.

**Editorial coordination**

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**Declaration of competing interest**

Doctors Naranjo, Tavera, Mancilla and Espitia have received conference fees from BioMarin Pharmaceuticals Inc. Dr. Hernández has no disclosures to make.

**Appendix A. Annex 1**

| Clinical phenotype | Atypical phenotype | Atypical phenotype | Atypical phenotype | Atypical phenotype | Atypical phenotype | Classical phenotype | Classical phenotype | Classical phenotype | Classical phenotype |
|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| ID #1              | Bogota Male       | Bogota Female     | Bogota Male       | Female             | Armenia Female    | Bogota Male       | Male               | 2                  | 3                  |
| Age (y)            | 6                  | 14                 | 10                 | 2                  | 16                 | 6                  | 5                  |                    |                    |
| Age at onset (y)   | 3                  | 10                 | 4                  | 2                  | 4                  | 2                  | 3                  | 2                  |                    |
| Age at first visit to pediatric neurology | 5                  | 12                 | 7                  | 2                  | 5                  | 2,5                | 3                  | 2,5                |                    |
| Affected relatives | Yes                | Yes               | Yes               | Yes               | No                 | No                 | Yes                | Yes                |                    |
| Initial symptoms   | Seizure           | Ataxia            | Language regression| Asymptomatic       | Language regression| Language regression| Seizure            | Global developmental delay |                    |

(continued on next page)
| ID  | Clinical phenotype | Atypical phenotype | Atypical phenotype | Atypical phenotype | Atypical phenotype | Classical phenotype | Classical phenotype | Classical phenotype |
|-----|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| #1  | Symptoms at first visit | Seizure, Abnormal movements and language abnormalities | Seizure, Abnormal movements and language abnormalities | Seizure, Abnormal movements and language abnormalities | Seizure, Abnormal movements and language abnormalities | Seizure, Abnormal movements and language abnormalities | Seizure, Abnormal movements and language abnormalities | Seizure, Abnormal movements and language abnormalities |
| #2  | Course of the disease before treatment | No changes | Symptoms seen on initial visit accompanied by abasia | Asymptomatic | Symptoms seen on initial visit accompanied by ataxia, abnormal movements and ophthalmological alterations. | Symptoms seen on initial visit accompanied by ataxia, abnormal movements and ophthalmological alterations. | Symptoms seen on initial visit accompanied by ataxia, abnormal movements and ophthalmological alterations. |
| #3  | Additional tests before treatment | EEG: No abnormalities, Brain MRI: No abnormalities, Visual potentials: Abnormal | EEG: Abnormal, Brain MRI: Abnormal | EEG: Abnormal, Brain MRI: Abnormal | EEG: Abnormal, Brain MRI: Abnormal | EEG: Abnormal, Brain MRI: Abnormal | EEG: Abnormal, Brain MRI: Abnormal |
| #4  | Tripeptidyl-peptidase 1 nmol/h/ml | Undetectable, Undetectable | <1.8, <1.6, 0.7 | Undetectable, 0.3, 0.6 | EEG with no changes | Brain MRI with no changes, EEG with no changes | Brain MRI with no changes, EEG with no changes, Abnormal Optic coherence tomography: Abnormal |
| #5  | Additional tests during treatment | EEG with no changes | Brain MRI with no changes, EEG with no changes. | Brain MRI with evidence of progression, EEG with no changes, Valproic acid and Lamotrigine | Valproic acid and Levetiracetam | Valproic acid and Levetiracetam and Clobazam | Valproic acid and Clobazam |
| #6  | Initial treatment | Valproic acid | Valproic acid and Levetiracetam | Valproic acid and Levetiracetam and Clobazam | Valproic acid and Clobazam | Valproic acid and Clobazam and Oxcarbazepine | Valproic acid and Clobazam |
| #7  | Valproic acid, Levetiracetam, and Lamotrigine | Valproic acid and Levetiracetam | Valproic acid and Levetiracetam and Clobazam | Valproic acid and Clobazam | Valproic acid and Clobazam and Oxcarbazepine | Valproic acid and Clobazam and Oxcarbazepine |
| #8  | Valproic acid, Levetiracetam, and Clobazam | Valproic acid and Clobazam | Valproic acid and Clobazam and Oxcarbazepine | Valproic acid and Clobazam and Oxcarbazepine | Valproic acid and Clobazam and Oxcarbazepine | Valproic acid and Clobazam and Oxcarbazepine | Valproic acid and Clobazam and Oxcarbazepine |

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