Congenital CLN8 disease of neuronal ceroid lipofuscinosis: a novel phenotype

Favio Pesaola, Romina Kohan, Inés A. Cismondi, Norberto Guelbert, Patricia Pons, Ana M. Oller-Ramírez, Inés Noher de Halac

Introduction. CLN8 disease is one of the thirteen recognized genetic types of neuronal ceroid lipofuscinosis, a group of neurodegenerative lysosomal storage disorders, most frequent in childhood. A putative 286 amino acids transmembrane CLN8 protein with unknown function is affected. Pathological variants in the *CLN8* gene were associated with two different phenotypes: variant late-infantile in individuals from many countries worldwide, and epilepsy progressive with mental retardation, appearing in Finnish and Turkish subjects.

Case report. The girl showed psychomotor delay and dementia since birth, tonic-clonic seizures, myoclonus, ataxia with cerebellar atrophy, and early death at 12 years old. Electron microscopy of the skin showed mixed GROD, curvilinear, fingerprint cytosomes and mitochondrial hypertrophy. Two pathological DNA variants in the *CLN8* gene (exon 2 c.1A>G; p.?/ exon 3 c.792C>G; p.Asn264Lys) were found confirming a compound heterozygous genotype.

Conclusion. This case is the Latin American index for a new congenital phenotype of the CLN8 disease. The congenital phenotype has to be added to the clinical spectrum of the CLN8 disease. The suspicion of CLN8 disease should be genetically sustained in challenging cases of a neurodegenerative syndrome with psychomotor delay since birth, speech difficulty and seizures. The course includes ataxia, cerebellar atrophy, and early death.

Key words. CLN8 disease. Compound heterozygous mutation. Congenital phenotype. Index case. Latin America. Neuronal ceroid lipofuscinosis.

Introduction

The CLN8 disease is one of the thirteen recognized genetic forms of neuronal ceroid lipofuscinosis (NCL), a group of inherited neurodegenerative lysosomal storage disorders. It is caused by DNA variants in the *CLN8* gene (MIM *607837) [1], which encodes for a putative 286 amino acids, endoplasmic reticulum-resident transmembrane protein (CLN8p) [2]. CLN8p role remains unknown; however, it was related with lipids [3]. Two phenotypes are related to DNA variants in the *CLN8* gene: epilepsy progressive with mental retardation (EPMR), also known as Northern epilepsy syndrome (OMIM #610003) [4] and a variant late infantile phenotype (vLI) (OMIM #600143) [5]. Main features of both variants are summarized in the table. None CLN8 disease case has been described so far in Latin America, and none with congenital, infantile or adult phenotypes.

This paper aims to resignify the value of a clinical-genomic approach in the clarification of a challenging case of a rare neurodegenerative disease from birth.

The procedures followed were in accordance with the International Declaration of Human Rights and Bioethics of UNESCO 2005, and the grandmother (caregiver) signed an informed consent approved by the Inter Institutional Committee of Ethics in Health Research (CIEIS–Cordoba, Argentina).

Case report

One of the Argentinean fraternal twin girls of non-consanguineous parents with a life span of 12 years. The mother died due to a cardiopathy and the father left the family some time before the consultation. She presented psychomotor delay and dementia since birth and tonic-clonic seizures since 3 years. She could walk alone at 2 years and she never could completely develop the speech. An MRI and CAT scan exams at 6 years revealed cerebellar atrophy (Fig. 1). At 7 years she displayed severe ataxia, constant tremor and falls and important neuromadurative delay, although she stayed connected with the caregivers. Neurometabolic studies carried out at CEMECO (qualitative plasma amino acids and urine oligosaccharides assays, and quantitative measures of urine organic acids) gave normal results, as well as the enzyme assays for palmitoyl protein thioesterase 1 (PPT1; EC 3.1.2.22) and tripeptidyl

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Table. Comparison of the natural histories of CLN8 congenital, vLI and EPMR.

		Congenital	vLI	EPMR
Origin		Argentina	Many countries worldwide	Finland and Turkey
Age at onset		Birth	2-7 years	5-10 years
Initial symptoms		Psycho-motor retardation, speech difficulties	Seizures, myoclonus or generalized psycho-motor impairment	Tonic-clonic seizures
Symptoms	Refractory seizures	Yes	Yes	Yes
	Myoclonus	Yes	Yes	No
	Motor impairment	Yes	Yes	Yes
	Intellectual disability	Yes	Yes	Yes
	Visual failure	?	Yes	No
Magnetic resonance imaging		Cerebellar atrophy	Cortical and cerebellar atrophy	Cortical and cerebellar atrophy
Light microscopy		No vacuolated lymphocytes	–	Globular neurons without vacuoles
Electron microscopy		Fingerprint bodies, curvilinear bodies, GRODs and mito-chondrial atrophy	Fingerprint bodies, curvilinear bodies and rarely GRODs	GRODs
Age at death		12 years	Generally, before 20 years	Generally, after 50 years
Pathological DNA variants stated		c.1A>G, p.? c.792C>G, p.Asn264Lys	Over than 30 mutations described	c.70C>G, p.Arg24Gly c.677T>C, p.Leu226Pro

EPMR: epilepsy progressive with mental retardation; GRODs: granular osmiophilic deposits; vLI: variant late infantile.

peptidase 1 (TPP1; EC 3.4.14.9). Intractable short seizures, myoclonus, atonic falls, osteotendinous hiperreflexia and inexhaustible spontaneous bilateral clonus in feet and hands were observed at 9 years. The skin biopsy revealed eccrine secretory gland cells with lipofuscin-like pigment accumulation grouped as granular osmiophilic deposits, fingerprint profiles and curvilinear bodies. (Fig. 2). These findings characterize NCL pathology.

Algorithm of diagnosis

The study is part of an observational cohort study of NCL occurrence in the Argentinean population. The diagnostic study of all the patients incorporated to the Translational NCL Research Program in Argentina is conducted following the previously published algorithm [6]. Methods for transmission electronic microscopy (TEM) and enzyme testing were published previously [6-9].

Genomic analyses

Genomic DNA was isolated from peripheral blood by Wizard DNA Purification Kit (Promega, USA) following manufacturer's protocol. *CLN8* coding region was amplified by PCR (primers available upon request). Sequencings were carried out at the University of Chicago Comprehensive Cancer Centre (Chicago, USA) and at the Neurogenetics DNA Diagnostic Laboratory of the Centre for Human Genetic Research (Massachusetts General Hospital, Boston, USA), both in an Applied Biosystems 3730XL sequencer. All wildtype sequences of *CLN8* gene, both human (RefSeq NM_018941, NP_061764) and other species, were obtained from Ensembl v. 90 database. DNA variants were excluded from 100 healthy individuals from the local population (samples kindly provided by the Blood Bank of National University Cordoba) using MEGA software v. 5.05 [10] for alignments. Non-synonymous protein variants were validated as with pathological significance by testing with SIFT [11], PolyPhen [12] and Mutation Taster softwares. Non-coding variants were analyzed using RESCUE-ESE software [13,14].

Sequencings showed the changes exon 2 c.1A>G and exon 3 c.792C>G, both in heterozygous state. Moreover, two other variants were observed in exon 1: c.-257G>C and c.-280_-279insG. The c.1A>G DNA variant was also found in heterozygous state in the twin sister and maternal grandmother, establishing that this DNA variant segregates from maternal line. The origin of the c.792C>G variant could not be stated due to the lack of paternal family samples.

Neither the c.1A>G nor the c.792C>G DNA variants were found in 100 controls of local population. The first methionine of CLN8p is highly conserved in all the species compared, unlike the asparagine in position 264. SIFT software predicted both mutations as 'damaging'; PolyPhen predicted them as 'probably damaging'; and Mutation Taster software predicted as 'disease causing' both variants. The variant exon 1 c.-280_-279insG could alter the predicted exonic splicing enhancer 5'-GCAGAA-3' spanning from -280 to -275. According with recommendations of the Human Genome Variation Society, the c.1A>G, p.? and c.792C>G, p.Asn264Lys mutations should be considered pathogenic significant. On the other hand, it could not be confirmed the pathogenicity of c.-280_-279insG and c.-257G>C DNA variants.

Figure 1. Magnetic resonance images of the patient at 6 years. It is observed a cystic formation at the posterior fossa that, as revealed by the sagittal plane, seems to be connected to the fourth ventricle, associated with the dilatation of the supratentorial ventricular system and it could be related to a variant of the Dandy-Walker syndrome.

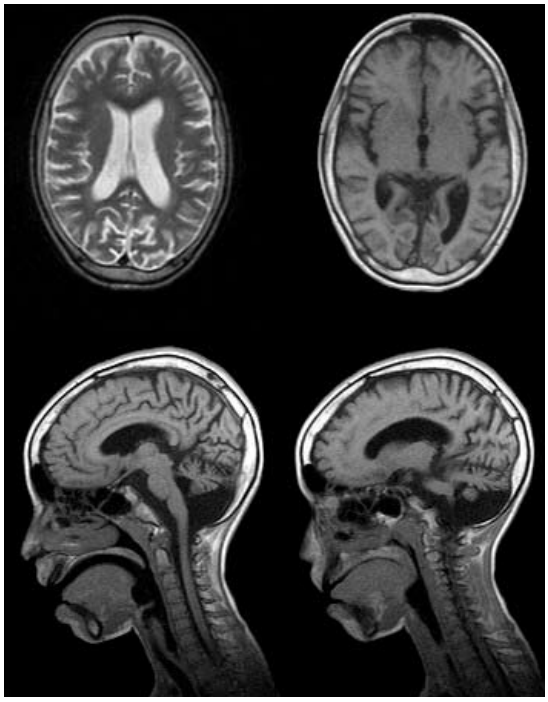
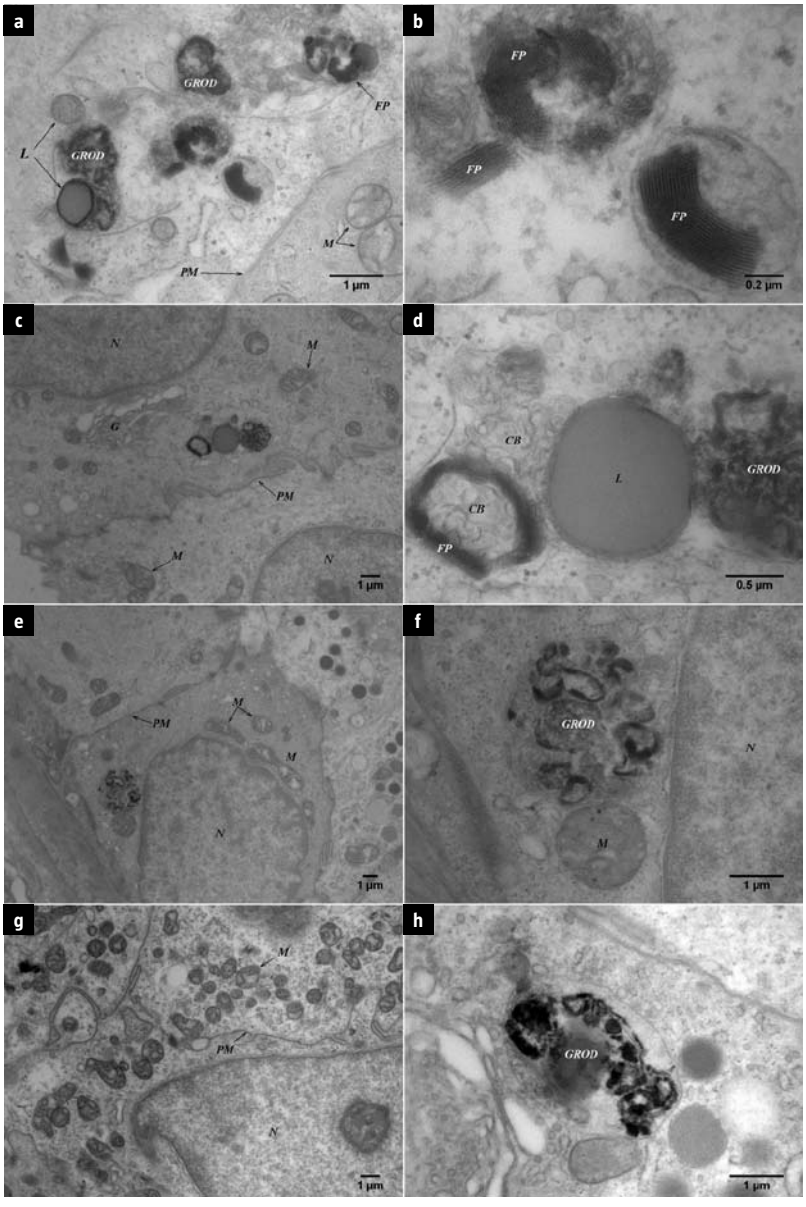


Figure 2. Transmission electronic microscopy (TEM) images of a skin biopsy. a, b) These images show some different accumulation profiles associated with lipid granules; hypertrophic mitochondria are frequently observed in TEM of CLN8 patients; c, d) Mixed profiles associated with lipid granules; e, f) Mixed profiles associated with hypertrophic vacuolated mitochondria; g) High number of hypertrophic mitochondria; h) Cytosome with a compound GROD accumulation. CB: curvilinear bodies; FP: fingerprint bodies; G: Golgi apparatus; GROD: granular osmiophilic deposits; L: lipid granule; M: mitochondria; N: nucleus; PM: plasma membrane.

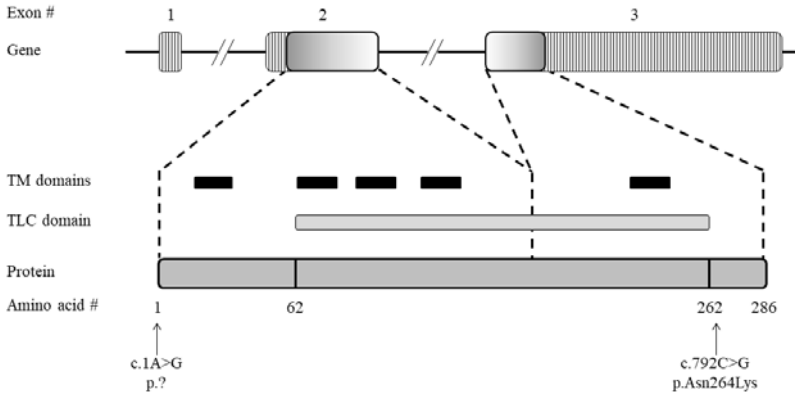


The DNA variant c.1A>G eliminates the first methionine codon; thus, it moves the translation origin downstream causing a frameshift, a novel stop codon and generating a 22 amino acids peptide. A subsequent BLAST analysis did not reveal a reliable homologous sequence for this polypeptide (unpublished data). According with mutation nomenclature of Human Genome Variation Society, this mutation should be coded as c.1A>G, p.?. Further analyses are necessary to confirm the expression of this apparently non-functional peptide. Both pathogenic variants were submitted to NCL-Resource, the principal database of NCL mutations, and ClinVar (accession no. SCV000676930 and no. SCV000660961).

Discussion

This girl showed a congenital phenotype with two heterozygous DNA variants with pathological significance (exon 2 c.1A>G, p.? [rs143730802] and exon 3 c.792C>G, p.Asn264Lys [rs587779411]) (Fig. 3), and other two with no pathological significance (exon 1 c.-257G>C [rs71499040] and exon 1 c.-280_-279insG [rs71209699]).

Figure 3. Scheme of *CLN8* gene and protein indicating the position of both pathogenic variants found in the patient of this study.



The natural history of the disease is summarized in the table in comparison with vLI and EPMR phenotypes. Clinical course was similar to the vLI form rather than EPMR, mainly due to its rapid evolution and the myoclonic movements. The visual aspects were not examined. The onset of symptoms at birth is sign of a congenital phenotype, that has to be added to the accepted late infantile and EPMR phenotypes of the CLN8 disease [15]. A congenital genotype/phenotype correlation could be explained by the lack of protein expression and malfunction. Up to date, CLN10 disease (OMIM #610127) was the only congenital NCL form [16]. The c.1A>G variant was cited previously in a CLN3 case (Batten disease, OMIM #204200) without a description of the patient's clinical course [17].

Both pathological significant variants are novel for the CLN8 disease. The exon 2 c.1A>G, p.? variant was reported previously in the *CLN8* gene in heterozygous state in four individuals as part of cohorts analyzed in massive sequencing projects: 1000 Genomes, UK 10K and NHLBI GO Exome Sequencing Project. However, the DNA variant was not described as related to CLN8 disease.

To our knowledge, this girl is the first Latin American case of CLN8 disease. Two CLN8 vLI siblings from Canada were reported. Another compound heterozygous case of *CLN8* (exon 2 c.1A>G, p.?/exon 2 c.80T>C, p.Leu27Pro) was diagnosed in USA. Two symptomatic individuals from Mexico and Argentina showed an heterozygous variant, c.685C>G, p.Pro229Ala, and were preliminary described as possible CLN8 disease subjects [18]. Further analyses revealed that this DNA variant is a frequent local polymorphism [19].

CLN8 vLI subjects described in the literature [5,20,21] showed delayed psychomotor development at early age (for example, start walking after 15 months or speaking at 3 years) and a progressive loss of mental and motor capabilities later, approximately after 3.5 years. All were diagnosed as vLI CLN8 disease, taking as the onset symptom the one that led to the medical consultation: unsteady gait, seizures, myoclonus or intellectual disability. A genotype/phenotype correlation in CLN8 disease was previously discussed [5]. In line with our findings, patients having congenital neurological symptoms should be grouped into a different phenotype: the congenital form of CLN8 disease.

Concluding, we identified the index congenital CLN8 case in Latin America caused by heterozygous DNA variants: a pathological (exon 2 c.1A>G, p.?) and a missense variant (exon 3 c.792C>G, p.Asn264Lys). The case expands the frontier of occurrence of CLN8 disease to Argentina and broadens information about the clinical spectrum of CLN8 disease, adding a congenital phenotype to be suspected in newborns with psychomotor delay since birth, speech difficulties, seizures syndrome with later myoclonic movement disorder and ataxia. The 'gene by gene' and 'exon by exon' PCR and Sanger sequencing methodology used in this case was time-consuming with high cost-effective ratio.

This study suggests that the systematic use of the analytical tools of new generation sequencing technology could speed up the recognition of challenging rare neurodegenerative disorders in newborns. The clarification of a rare neurodegenerative disease since birth by clinical-genetic analyses, can be postulated as the most suitable approach, to increase reading coverage reducing time-cost and diminishing the diagnostic odyssey of the families.

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Enfermedad CLN8 congénita de lipofuscinosis neuronal ceroidea: un nuevo fenotipo

Introducción. La enfermedad CLN8 es uno de los 13 tipos genéticos reconocidos de lipofuscinosis neuronal ceroidea, un grupo de trastornos neurodegenerativos de acumulación lisosómica, los más frecuentes en la infancia. La causan mutaciones en la proteína transmembrana CLN8 de 286 aminoácidos, cuya función se desconoce. Las variantes patológicas en el gen *CLN8* se asociaron con dos fenotipos diferentes: la variante infantil tardía en individuos de diversos países alrededor del mundo, y la epilepsia progresiva con retraso mental, que aparece en pacientes finlandeses y turcos.

Caso clínico. Niña que mostró retraso psicomotor y demencia desde el nacimiento, convulsiones tonicoclónicas, mioclonía, ataxia con atrofia cerebelosa y muerte temprana a los 12 años. La microscopia electrónica de la piel mostró una mezcla de citosomas con patrones de depósitos osmiofílicos granulares, curvilíneos y de 'huella digital', y mitocondrias hipertrofiadas. Se encontraron dos variantes patológicas de ADN en el gen *CLN8* (exón 2 c.1A>G; p.?/ exón 3 c.792C>G; p.Asn264Lys), lo que confirmó un genotipo heterocigoto compuesto.

Conclusión. Éste es el caso índice en América Latina para el nuevo fenotipo congénito de la enfermedad CLN8. La sospecha de esta patología debería sustentarse genéticamente en casos de síndrome neurodegenerativo con retraso psicomotor desde el nacimiento, dificultad del habla y convulsiones. El curso clínico incluye ataxia, atrofia cerebelosa y muerte temprana.

Palabras clave. América Latina. Caso índice. Enfermedad CLN8. Fenotipo congénito. Lipofuscinosis neuronal ceroidea. Mutación heterocigota compuesta.