Original Article

Neuronal Ceroid Lipofuscinoses in Children

Mahesh Kamate, Narendranadha Reddy[1], Mayank Detroja[2], Virupaxi Hattiholi[3]

Division of Pediatric Neurology and In-Charge Child Development Clinic, Department of Pediatrics, KAHER's J N Medical College, Departments of [1]Pediatrics and [3]Radiology, KAHER's J N Medical College, [2]Department of Child Development and Pediatric Neurology Centre, KLES PK Hospital, Belgaum, Karnataka, India

Abstract

Background: The neuronal ceroid lipofuscinoses (NCL) constitute a group of gray matter neurodegenerative disorders characterized by the accumulation of ceroid lipopigment in lysosomes in neurons and other cell types. There are very few published studies on NCL from India, especially in children. **Methods:** A retrospective study of confirmed patients of NCL diagnosed over a period of 10 years from January 2019 to December 2019. **Results:** Fifty children had a definitive diagnosis of NCL based on enzymatic studies or genetic testing using next-generation sequencing. Around 15 children were diagnosed to have CLN-1 (ceroid lipofuscinoses, neuronal-1) based on palmitoyl protein thioesterase-1 deficiency; 24 children were diagnosed with CLN2 (ceroid lipofuscinoses, neuronal-2) based on deficient tripeptidyl-peptidase-1 activity; three patients were diagnosed as CLN6, five patients as CLN7, one case each of CLN8, CLN11, and CLN14 based on genetic testing. Clinical presentation was quite varied and included refractory seizures, developmental delay/regression, and abnormal movements. Visual failure was not common in the present case series. Neuroimaging patterns in different types of NCL were different. All children had a progressive downhill course resulting in death in many over a period of 5–10 years of disease onset. **Conclusion:** NCL is not uncommon and diagnosis can be suspected based on clinical investigations and neuroimaging findings. Diagnosis can be confirmed by enzymatic assays or genetic testing.

Keywords: Enzyme assay, neuroimaging, neuronal ceroid lipofuscinoses, next-generation sequencing

Neuronal ceroid lipofuscinosis (NCL) is a group of neurodegenerative disorders mainly affecting the gray matter characterized by seizures, cognitive decline, myoclonus, visual impairment, and abnormal movements.[1] These disorders are both clinically and genetically very heterogeneous, thereby complicating its diagnoses. They are characterized by lysosomal accumulation of autofluorescent material in neurons and extraneuronal tissues with an incidence of 1 in 12,500 to 1 in 100,000 births in the west.[1-4] There are no epidemiological studies from India. The age of symptom onset varies widely from infancy to late adulthood; mostly adult case series dominate the literature. Neuroimaging abnormalities in NCL include varying degrees of diffuse cerebral atrophy, cerebellar atrophy, and thalamic hypointensities.[5] The diagnosis is established on the basis of electron microscopy of skin biopsy, enzyme assay for few types of NCL, and by genetic testing mainly using next-generation sequencing (NGS) techniques. Although they are the most common type of progressive myoclonus epilepsies seen in children, comprehensive studies from the Indian subcontinent describing a large cohort of NCL are limited. This often results in delay in diagnosis, failed opportunity for prenatal diagnosis, and stress on the parents. Only a few case series of NCL in children are published from India and they are mainly based on enzymatic studies.[6] The published large studies on NCL from India were mainly based on electron microscopy.[7,8] With the commercial availability of NGS, we are able to diagnose different types of NCL. Enzyme replacement therapy is being tried for CLN2 patients and animal studies are on for a few other types of NCL.[9-11] Hence, it is important to know the different types of NCL, their presentation, and the natural history of the disease. The purpose of this study is to describe our experience with pediatric NCL in a tertiary care center in southern India. Herein, we report the clinical findings, correlate the neuroimaging findings with the type of NCL, and depict the natural history of the disease.

Methods

A retrospective review of files of children with a diagnosis of neurometabolic/neurodegenerative disorders in the last 10 years was done. Only those, where the diagnosis of NCL was confirmed by genetic analysis (NGS) and/or enzyme assays were taken for the final analysis. Lysosomal enzyme study was carried out from leukocytes using 4-MU specific substrate for PPT1 and TPP1 for NCL type-1 (CLN1) and NCL type-2 (CLN2), respectively. The presenting features including details of developmental milestones, family history, neurological examination details, neuroimaging, and electrophysiology findings of all patients with NCL were

Address for correspondence: Prof. Mahesh Kamate, Professor of Paediatric Neurology, KAHER's J N Medical College, Belgaum - 590 010, Karnataka, India.
E-mail: drmaheshkamate@gmail.com

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extracted and entered in predesigned proforma. Children who were alive were called for follow-up, and their current status was noted. Siblings when available were also examined. Among those who died, the age of death was recorded. The details of the genetic defect and enzyme assays were also noted. Information of some patients who were lost to follow-up was obtained by telephonic interview. Descriptive statistical methods were applied for analyzing the data. Categorical variables were presented as frequency percentage. Continuous variables were presented as mean and standard deviation if normally distributed and as median and range otherwise.

Results

A total of 50 children had genetically/enzymatically confirmed NCL [Tables 1 and 2] and were diagnosed over the last 10 years. Because of the cost and ease of availability, most cases of NCL-1 and NCL-2 were diagnosed based on enzymatic assays and other types were diagnosed based on NGS gene panels. Fifteen children were diagnosed to have CLN-1 (ceroid lipofuscinosis, neuronal-1) based on palmitoyl protein thioesterase-1 (PPT1) deficiency; 24 children were diagnosed with CLN2 (ceroid lipofuscinoses neuronal-2) based on deficient tripeptidyl-peptidase-1 (TPP-1) activity; three patients were diagnosed as CLN6, five patients as CLN7, one case each of CLN-8, CLN-11, and CLN-14 based on NGS.

CLN1/palmitoyl protein thioesterase-1 (PPT1) related NCL

The key clinical findings and investigations of patients diagnosed with PPT1 related NCL are summarized in Table 1. Common presenting features included motor delay/regression, abnormal movements, and irritability. Seizures were present in only nine patients (60%); none had microcephaly at presentation. Parents did not have any visual concerns and fundus examination was normal in all patients. Consanguinity was present in 11 children (73.3%). The median time taken for diagnosis was 24 months (range: 18–48 months). All patients had deficient PPT1 enzyme activity. The median duration of follow-up was 68 months (range: 36-108 months) and all of them had a progressive downhill course. The MRI of the brain in all patients showed diffuse cerebral atrophy with thalamic hypointensities [Figure 1a and b]. The cerebellum was normal at presentation. Follow-up imaging showed progressive cerebral atrophy. The electroencephalogram in all patients showed background slowing with occasional generalized sharp waves in few. About 12 of 15 children died after a median age of 5 years of the disease.

CLN 2/Tripeptidyl-peptidase-1 (TPP1) related NCL

There were 24 cases of TPP1 related NCL, the most common type in our series. The median age at presentation was 4 years 2 months (range: 6 months to 8 years). The clinical findings and neuroimaging findings are summarized in Table 1. The most common presenting feature was seizures, which was seen all patients, followed by regression of milestones. Seizures were of complex partial, myoclonic, or generalized tonic-clonic (GTCS) types. One of the patients presented with delayed attainment of milestones, chorea-athetosis, and reflex (auditory) myoclonus. None of the patients complained nor their parents had any visual concerns and two patients had partial optic atrophy on fundus examination. All of them had cerebellar atrophy and periventricular white matter hyperintensities on MRI and in those who also had cerebral atrophy; cerebellar atrophy was more pronounced than the cerebral atrophy [Figure 1c and d]. In two patients who had serial neuroimaging done, the cerebellar atrophy was progressive. All patients had a significantly deficient activity of TPP enzyme. All patients had a progressive worsening in the seizures and ataxia. Eleven of 24 patients became nonambulatory 1–3 years after diagnosis (mean age 9 years; SD: 5.92). There was a delay of 6 to 24 months before reaching the correct diagnosis. Four patients had received a diagnosis of the mitochondrial disorder and were given treatment for the same before NCL was diagnosed.

Other CLN types

CLN7 was the next common type of NCL seen in five children. The median age at presentation was 3 years (range: 2–4 years) and consanguinity was seen in four of seven families. Two were siblings. All had seizures, cognitive regression, myoclonic seizures, and ataxia. One child had opsoclonus also and fundus examination was normal in all children. EEG showed

Table 1: Table showing salient clinical manifestations, age at presentation/diagnosis, neuroimaging findings, and survival details in children with CLN1 and CLN2 disease

Characteristic	CLN 1	CLN 2
Number of patients	15	24
Consanguinity	11/15 (73.3%)	9/16 (56.3%)*
Refractory seizures	13/15 (86.6%)	14/20 (70%)
Regression of milestones/dementia	15 (100%)	24 (100%)
Ataxia/myoclonus	9/15 (60%)	11/20 (55%)
Age at presentation Median (range)	12 months (6 to 30 months)	50 months (6 to 96 months)
Age at diagnosis Median (range)	24 months (18 to 48 months)	64 months (24 to 144 months)
MRI imaging	Prominent cerebral atrophy with hypointense thalami in all 15 patients	Predominant cerebellar atrophy with periventricular hyperintensities in all
Age at death (median and range)	68 months (36 to 108 months) (12/15), (3 patients are alive)	108 months (72 months to 20 years)**

*data was not available in 8 children with CLN2 disease.** data was available only for 14 children with CLN2 disease

Table 2: Table showing clinical, neuroimaging, genetic defect, duration of disease, and survival of NCL patients other than CLN1 and CLN2

Age of onset/Sex	Clinical features	Examination Findings	Consan	Neuroimaging	Type/NGS findings	Outcome/Duration of illness
5 year/F	Regression of milestones, ataxia, hyperactive behaviour, decreased speech output	Myoclonus microcephaly	No	Thalamic hypointensities, periventricular WM changes with mild cerebellar atrophy	CLN6/Homozygous c.185G >A inexon2 of *CLN6* gene	Alive, 3 years
4 year/M	Regression of milestones, refractory seizures	Myoclonus, spasticity	III	Thalamic hypointensities, periventricular WM hyperintensities and mild cerebellar atrophy	CLN 6/Homozygous c.476C >T in exon 4 of *CLN6* gene	Died at 11 years of age
5 year/M	Regression of milestones, refractory seizures, frequent falls	Microcephaly, ataxia, spasticity in limbs	III	Thalamic hypointensities, periventricular WM changes with mild cerebellar atrophy	CLN 6/Homozygous c.184C >Tin exon 2 of *CLN6* gene.	Alive at 14 years, bedbound at present
2 year/M	Regression of milestones decreased speech, abnormal movements	Myoclonus, fundus normal	No	Thalamic hypointensities, periventricular WM changes, prominent cerebellar atrophy	CLN7/Homozygous c.694delC in exon 7 of *MFSD8* gene	Died at 7 years
3 year/M	Seizures-3 years, abnormal jerks-4 years, motor regression-5 years	Opsoclonus, myoclonus	II	Thalamic hypointensities, periventricular WM changes with severe cerebellar atrophy	CLN7/Homozygous c.699-1G >A in intron 7 of the *MFSD8* gene	Bedridden at 6 years
2 year/M	Seizures, regression of milestones and abnormal eye and limb movements	choreoathetoid movements, ataxia, microcephaly	II	Thalamic hypointensities, periventricular white matter changes with diffuse cerebellar atrophy	CLN7, Homozygous c,699-1G >A in essential splice acceptor site in intron 7 of the *MFSD8* gene	Alive, bedridden at 5 years
4 year/M	Seizures, regression of milestones, imbalance while walking, myoclonic jerks	myoclonus	III	Thalamic hypointensities, periventricular white matter changes with diffuse cerebellar atrophy	CLN7/Homozygous c.699-1G >A in intron 7 of the *MFSD8* gene	Alive, no improvement
3 year/M	Seizures, regression, and abnormal movements	Choreoathetoid movements,	III	Thalamic hypo intensities, mild diffuse cerebellar atrophy with periventricular white matter changes	CLN 7 Homozygous 5' splice site variation in intron 3 c 154 + 1G >A of *MFSD8* gene	Died at 9 years of age
2 year/M	Seizures, regression of milestones, imbalance while walking	Ataxia	III	Thalamic hypointensities, periventricular white matter changes with mild diffuse cerebral and cerebellar atrophy	CLN 8/Homozygous c.1A >C in exon 2 of the *CLN8* gene.	Bedbound at 9 years of age
12 year/F	Recurrent seizures	Mild gait ataxia, poor short-term memory	II	Diffuse vermian and cerebellar atrophy with hypoplasia	CLN11/Homozygous progranulin gene (*GRN*) in exon 9 c. 912G >A position	Seizures reasonably under control
3 year/F	Delayed speech, choreoathetoid movements and seizures	myoclonic jerks, choreoathetoid movements, autistic features	II	Generalized cerebellar atrophy	CLN14/Homozygous dupc.(314+1_315-1)_(*3997_?) duplication of exons 3-4 in the *KCTD7* gene.	Alive, no improvement

Consang: Degree of consanguinity; WM: White matter; NGS: Next-generation sequencing

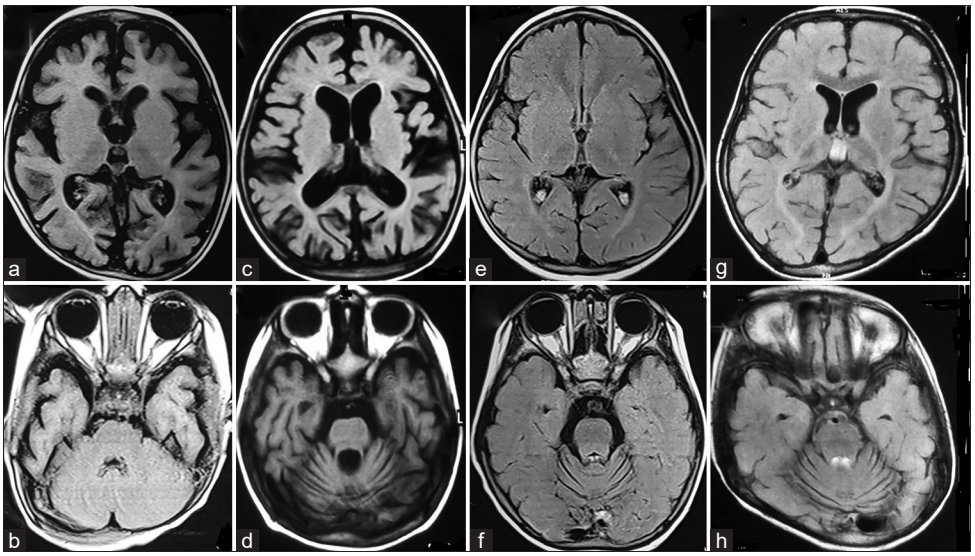

Figure 1: (a, b) FLAIR axial section of the brain of CLN1 child at the level of basal ganglia and cerebellum respectively showing diffuse cerebral atrophy, thalamic hypointensities, and sparing of the cerebellum. (c, d) FLAIR axial section of the brain of CLN2 child at the level of basal ganglia and cerebellum respectively showing diffuse cerebellar and cerebral atrophy (cerebellum more than cerebrum); normal thalamus and periventricular white matter hyperintensities. (e, f) FLAIR axial section of the brain of CLN6 child at the level of basal ganglia and cerebellum, respectively showing mild diffuse cerebellar atrophy, thalamic hypointensities, and periventricular white matter hyperintensities. (g, h) FLAIR axial section of the brain of CLN7 child at the level of basal ganglia and cerebellum, respectively showing prominent diffuse cerebellar more than cerebral atrophy, thalamic hypointensities, and periventricular white matter hyperintensities

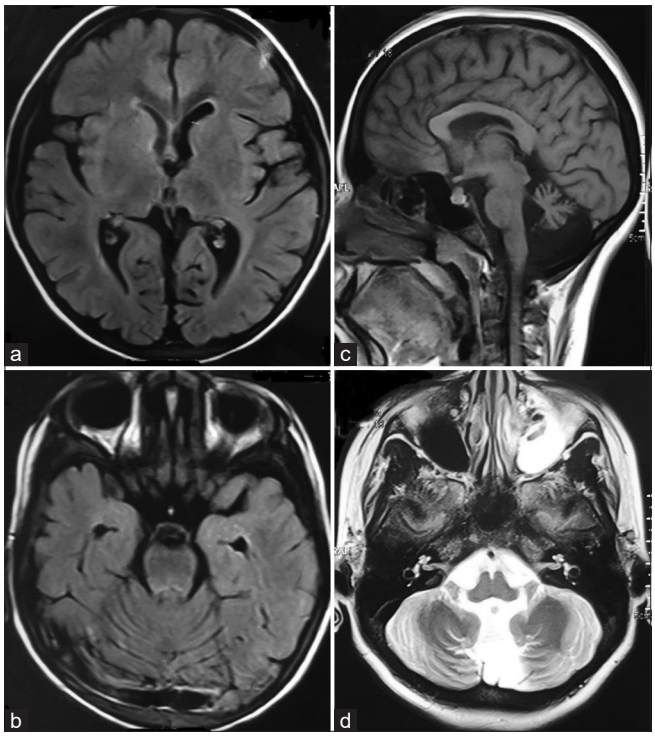

Figure 2: (a, b) FLAIR axial section of the brain of CLN8 child at the level of basal ganglia and cerebellum, respectively showing mild diffuse cerebellar and cerebral atrophy, thalamic hypointensities, and periventricular white matter hyperintensities. (c, d) T1-weighted sagittal section of the brain and T2-weighted axial section of the brain at the level of cerebellum respectively showing isolated cerebellar atrophy (mainly vermian) with hypoplasia

diffuse background slowing with occasional generalized sharp waves. MRI brain showed early prominent cerebellar atrophy, periventricular white matter hyperintensities, and thalamic hypointensities in all [Figure 1e and 1f]. In three children where follow-up imaging was available after a year of disease onset, there was progressive cerebellar atrophy with diffuse cerebral atrophy, periventricular hyperintensities, and thalamic hypointensities. Three children had CLN6. All had onset less than 5 years and presented with seizures, dementia, myoclonic jerks, and seizures. Two were born to a consanguineous couple. The MRI of the brain in all patients showed thalamic hypointensities and periventricular white matter hyperintensities like in CLN7 but the cerebellar atrophy was very mild. The only child with CLN8 had a similar clinical presentation at 3 years and his MRI showed thalamic hypointensities with periventricular white matter hyperintensities but both cerebral and cerebellar atrophy [Figure 1g and 1h]. We had one child with CLN11 who presented at 12 years of age with gradually increasing frequency of seizures for the past 1 year without any signs of visual abnormalities and dementia. She had mild gait ataxia. Her electroencephalogram showed frequent generalized epileptiform discharges and magnetic resonance imaging (MRI) showed diffuse vermian and cerebellar atrophy with hypoplasia [Figure 2a and 2b]. Her seizures were well-controlled with antiepileptic drugs and she continued to attend school even 2 years into the disease suggesting a mild disease. The only case with CLN14 in our series presented with developmental delay and dyskinesias in the form of choreoathetosis and dystonia along with myoclonus. She also had autistic features. Her EEG showed hypsarrhythmia like pattern. Her MRI showed only generalized cerebellar atrophy without hypoplasia, periventricular white matter hyperintensities, and thalamic involvement [Figure 2c and 2d].

There was second-degree consanguinity and her genetic tests revealed duplication in *KCTD* gene suggesting CLN14. With steroids, her myoclonic jerks reduced in frequency and currently she has only 3–4 myoclonic jerks per day in isolation. Almost 5 years into the disease she is still surviving with myoclonic jerks and autistic behavior and there is no regression of any attained milestones suggesting a mildly progressive course.

Discussion

Diagnosing NCL in pediatric cases can be challenging in view of varying age of onset, clinical features, neuroimaging features, and different types of NCL described. Delayed or missed diagnosis deprives the parents of prenatal diagnosis and hence the urgency in reaching the correct diagnosis. NCL has been traditionally classified based on the age of onset, clinical features, and histopathological findings into infantile, late infantile, juvenile, and adult forms. It is now known that NCLs exhibit remarkable genotype-phenotype heterogeneity.[1,4] Identical phenotypes can result from different mutations in a single gene or different mutations in different genes. This was seen in our study also, with CLN2 patients presenting anywhere from 6 months to 8 years of age. Traditionally, CLN2 was to have a late-infantile presentation. Similarly, the same mutation in a single gene may result in variable phenotype among individuals, and within a single-family. Hence, the nomenclature based on the genetic/enzymatic defect is now preferred over/along with phenotypic classification.

Out of 50 cases of confirmed NCL, the most common type of NCL was CLN2 followed by CLN1. This was followed by CLN7. This could be due to the fact that CLN1 and CLN2 can be easily diagnosed by enzymatic assay which is cheaper than the genetic studies and the genetic studies became widely available and affordable only in the last 5 years. There was no gender predisposition and there was variable age of onset ranging from less than 6 months to adolescent age group though most of them presented in the first 5 years of age. Most of them had a progressive decline in cognitive functions but not all of them had seizures. CLN11 and CLN14 patients had a more slowly progressive course as compared to other types. Many of them had abnormal movements such as myoclonus, dyskinesia, and ataxia. Because of the young age of cases, many of them did not have visual complaints and fundus examination in many was normal at presentation.

Neuroimaging in the form of MRI was useful in suggesting the diagnosis of NCL. Careful correlation of the findings with the type of NCL gave important patterns that could suggest a particular type of NCL and aid further testing in the form of enzymatic studies or genetic diagnosis. While in all CLN1 children there was early prominent cerebral atrophy with thalamic hypointensities and sparing of the cerebellum at presentation, in all CLN2 children there was diffuse early cerebellar atrophy with faint periventricular white matter hyperintensities. The imaging findings of CLN6 and CLN7 overlapped with both types showing thalamic hypointensities, periventricular white matter hyperintensities, and cerebellar atrophy; the CLN7 patients had more pronounced and early cerebellar atrophy. In other types of NCL, as there was the single case only, it is difficult to generalize however, the pattern appeared different as compared to other types. More studies on this aspect from other centers involving more patients can help to confirm these findings. The suggested MRI findings are relevant in the initial part of the disease i.e. in the initial few months of presentation. As the disease progressed, all types had diffuse cerebral and cerebellar atrophy with periventricular white matter hyperintensities and the imaging pattern was not helpful in suggesting a particular type of NCL at later stages of the disease.

Knowing the specific type of NCL helps in knowing the natural history of the disease, to counsel parents about the treatment prospects, and prenatal diagnosis can be offered. Enzyme replacement therapy is being tried in children with CLN2 and animal studies are going on for CLN1 and CLN10. This highlights the importance of knowing the type of NCL in the current era.[9-11]

An important limitation of the current study is its retrospective design. Patients who were bedbound and from far-off places did not come for follow-up. Electrophysiological studies (ERG, VEP) and electron microscopy of skin biopsy, lymphocytes for inclusion bodies were not done because of lack of facilities in the study center. Multicenter collaborative prospective studies can overcome these limitations in future.

To conclude, this study confirms the genetic, neuroradiological, and clinical heterogeneity of NCL in children. Neuroimaging findings can give a clue to the underlying type of NCL. While type-1 and type-2 can be diagnosed by enzyme assay, diagnosis of other types need genetic studies using NGS gene panels.

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Conflicts of interest

There are no conflicts of interest.

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