Neuronal Ceroid Lipofuscinosis: Clinical and Laboratory Profile in Children from Tertiary Care Centre in South India

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Abstract

Neuronal ceroid Lipofuscinosis (NCL), inherited disorders of lysosomal storage disorders, constitute the most common progressive encephalopathies with an incidence of 1.3 to 7 in 100,000 live births. We reported clinical, electrophysiological, radiological, ultrastructural, and molecular genetic features of NCL. This is a retrospective review, in a tertiary care center from January 2016 to December 2019. All children with clinical features of NCL and confirmed by pathogenic mutation and/or enzyme assay were included. A total of 60 children (male:female = 3:1) were studied. The commonest type was CLN 2 (41.7%). Neuroregression, seizures, and ataxia were present in all cases. Retinal arterial attenuation was seen in 38.33% cases. Magnetic resonance imaging (MRI) brain was abnormal in all patients, thalamic and caudate nucleus atrophy common in CLN1 (62%). Electroencephalography was abnormal in all children, but photoparoxysmal response at low intermittent photic stimulation frequencies was seen in four children of CLN2. Electron microscopy done in 43 children revealed abnormal inclusions in 20 (46.52%) children. Enzyme study showed low levels in 36 (78%) out of 46 cases. Of these, 21 had low tripeptidyl peptidase and 15 had low palmitoyl protein thioesterase levels. Molecular testing done in 26 cases showed pathogenic variant in 23 (88%) cases. Infantile onset with thalamic atrophy on MRI is common in CLN1 and refractory epilepsy, visual impairment and specific EEG changes are common in CLN2. These features are helpful in selecting enzyme assay for CLN1 versus CLN2. Electron microscopy helped in the diagnosis and genetic testing in subtyping. Thus, a multimode approach played a role in the diagnosis of NCL.

Keywords
- cerebellar atrophy
- CLN genetic loci
- involuntary movements
- myoclonic epilepsy
- neuronal ceroid lipofuscinoses
- neuroregression

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Introduction

Neuronal ceroid lipofuscinosis (NCL) is a group of autosomal recessive inherited lysosomal neurodegenerative diseases characterized by the intracellular accumulation of fluorescent lipopigments, ceroid, and lipofuscin that causes progressive neurologic degeneration.[1] The clinical course includes progressive dementia, seizures, movement abnormalities, and progressive visual failure. Incidence ranges from 1.3 to 7 in 100,000 live births and varies among geographic ethnic regions.[1] The term "NCL" was coined by Zeman et al to distinguish the familial cerebromacular degeneration clinically and pathologically from gangliosidosis.[2] Based on age at onset, six basic subtypes, consisting of congenital (CLN10), infantile (CLN1), late infantile (CLN2), variant late infantile (CLN5, CLN6, CLN7, and CLN8), juvenile (CLN3), and adult (CLN4 or Kufs disease) has been reported.[3] However, there is variation in age of onset and disease progression among the various groups posing difficulty in categorizing to a particular subtype. In the recent past, with the advent of molecular diagnosis and identification of causative genes, a novel NCL nomenclature has been proposed taking into consideration the mutation with key clinical and pathological features. Thus, in the novel nomenclature, the gene mutated denotes the specific subtype, followed by the clinical presentation based on symptomatology, age of onset, and EM findings, (e.g., CLN2 disease and late infantile type).[4] Currently, 14 NCL gene loci are identified.

The NCL is often diagnosed late due to lack of awareness and clinical features are not specific. Over the past decade, there have been improvements in the understanding of the underlying pathophysiology of NCLs, development of reliable animal models, and therapeutic success in these animal models. In April 2017, Food and Drug administration approved cerliponase alfa recombinant human tripeptidyl peptidase (TPP)1 for the treatment of NCL2, which is an innovative treatment available for this rare disease.[5] Hence, early diagnosis is important. The NCLs share common clinical features such as epilepsy, neuro-regression, visual impairment, and premature death. It is imperative to gain adequate information of various subtypes, clinical, and laboratory parameters as treatments become available. Hence, this study was planned.

Methods

This is a retrospective review of all cases diagnosed with NCL in a tertiary care center, Bengaluru, India from January 2016 to December 2019. All children with clinical features of NCL and confirmed gene mutation and/or enzyme assay were included. Detailed clinical history, examination, and investigations which include MRI of brain, skin biopsy, ophthalmological examination, enzyme assay, and molecular analysis were taken from case records in all patients.

For enzyme assay, the whole blood samples (4 mL) were collected in sodium heparin and subjected to hemolysis by the treatment with a hypotonic solution to obtain a leukocyte pellet. The leukocyte pellet is sonicated under the ice to make a protein homogenate. The protein content of this homogenate was measured by bicinchoninic acid assay where in the protein-based reduction of Cu^{+2} to Cu^{+1} in the alkaline solution is measured. For TPP assay, the protein homogenate at 6× dilution was incubated with 100 µL 0.25 mM Ala-Ala-Phe 7-amido-4-methylcoumarin substrate in 75 mM sodium acetate buffer, pH 4.0 for one hour at 37°C. The reaction is quenched by addition of 150 µL of 0.2 M sodium phosphate/0.1 M citrate buffer, pH 4.5. Tritonx-100 was present in both the buffers at a concentration of 0.025% w/v to serve as surfactant. Using the standard 7-amido-4-methylcoumarin fluorescence as a reference, the fluorescence was measured at 440 nm and accordingly enzyme activity is expressed and activity less than 10% was taken as abnormal. For palmitoyl protein thioesterase assay, the protein homogenate (10 µL) was incubated with 20 µL substrate (0.64 mM methylumbelliferyl-6-thio-palmitoyl-β-D-glucoside, including β glucosidase, triton X 100 + DTT/BSA in 0.2 M sodium phosphate/0.1 M citrate buffer, pH 4.0) for one hour at 37°C. The reaction is quenched by the addition of 200 µL carbonate buffer (0.5 M sodium carbonate buffer, pH 10.7 with 0.025% triton x-100). Enzyme activity was calculated based on a calibration curve of methylumbelliferone standard and activity less than 10% was taken as abnormal.

Genetic testing was done by targeted gene sequencing and confirmed by Sanger sequencing. DNA extracted from blood was used to perform targeted gene capture using a custom capture kit. The libraries were sequenced to mean >80 to 100× coverage on illumina sequencing platform as per the manufacturer protocol. Sequences obtained were aligned to the human reference genome (GRCh37/hg19), QC, data mapping, variant calling, and annotation of variants with external and internal data sources were achieved with a customized genome Analysis Toolkit framework. Gene/variant annotation was achieved using the variant effect predictor program[6] against the ensemble release 91-human gene model.[7] Each transcript listed, the analyzed region includes coding exons and ± 10 base pair of flanking intronic region on both sides of each exon. Clinically, relevant mutations were annotated using published variants in literature and a set of diseases databases— ClinVar, OMIM (updated on November 21, 2018), GWAS, HGMD (v2018.3), and SwissVar.[8–12] Common variants are filtered based on allele frequency in 1000Genome Phase 3, ExAC (v1.0), gnomAD (v2.1), EVS, dbSNP (v151), 1000 Japanese Genome, and Indian population database.[13–16] Nonsynonymous variants effect was calculated using multiple algorithms such as PolyPhen-2, SIFT, MutationTaster2, and LRT. Only nonsynonymous and splice site variants found in the targeted gene capture were used for clinical interpretation and synonymous, and deep intronic variants without being reported elsewhere were not considered for the analysis. QIAGEN Ingenuity Variant Analysis was used to identify variants which are relevant to the clinical indication and were classified as per the American College of Medical Genetics and Genomics (ACMG) guidelines.[17] The variants were classified based on the standard guidelines of ACMG/Association for Molecular Pathology, and are scored based on the evidence and strength of each criteria as described in the ACMG guidelines. The detailed description

Table 1 Various subtypes and age of presentation of neuronal ceroid lipofuscinoses

CLN types	Number of children $n = 60$ (%)	Mean age of presentation	Range of presentation
CLN 1	21 (35)	9.9 months	2 months to 3.4 years
CLN 2	25 (42)	2 years 10 months	0 to 7 years
CLN 3	01 (02)	7 years	7 years
CLN 5	01 (02)	7 years	7 years
CLN 6	05 (08)	4 years 2 months	3 to 5 years
CLN 7	03 (05)	2 years 3 months	2 to 7 years
CLN 8	03 (05)	3 years 2 months	18 months to 4 years
CLN 11	01 (02)	13 years	13 years

of ACMG criteria for classifying pathogenic variants are mentioned in the supplementary document.

Based on the clinical, enzyme, and molecular analysis, the patients were categorized into various subtypes. The study was approved by the institutional ethical committee.

Results

A total of 60 patients with diagnosis of NCL were analyzed. There was male preponderance of 68.33%. Out of 60 patients, enzyme assay was done in 46 patients, mutation analysis was done in 26 patients, and electron microscopy in 43. The various subtypes and age of presentation is depicted in ►**Table 1**. Detailed clinical features and laboratory findings are mentioned in ►**Table 2**. Consanguineous marriage was noted in 80% with a positive family history of siblings affected or death due to similar complaints in seven. History of seizures and neuroregression, ataxia was present in all

Table 2 Various clinical and laboratory features of neuronal ceroid lipofuscinoses

Parameter	CLN1 $n = 21$ (35%)	CLN2 $n = 21$ (41.66%)	CLN 6 $n = 5$ (8%)	CLN7 $n = 3$ (5%)	CLN8 $n = 3$ (5%)
Mean age of presentations	9.9 months	2 years 10 months	4 years 2 months	2 years 3 months	3 years 2 months
Developmental delay	12 (57)	16 (64)	3 (60)	2 (66)	2 (66)
Regression	21 (100)	25 (100)	5 (100)	3 (100)	3 (100)
Abnormal eye findings	15 (71)	23 (92)	2 (40)	3 (100)	2 (66)
Epilepsy	21 (100)	25 (100)	5 (100)	3 (100)	3 (100)
Myoclonic epilepsy	18 (86)	25 (100)	5 (100)	3 (100)	3 (100)
Involuntary movements	21 (100)	25 (100)	5 (100)	3 (100)	3 (100)
Electroencephalography					
PPR at low IPS frequencies of 1 to 3 Hz	0	04 (16)	0	0	0
Periodic discharges	0	1	0	0	1
Multifocal epilepsy	13 (21)	20(33)	4(6)	2(3)	2(3)
Magnetic resonance imaging of brain					
Thalamus	10 (47)	2 (8)	0	0	01 (33)
Caudate	13 (62)	2 (8)	0	0	0
Cerebellar and cerebral atrophy	21 (100)	25 (100)	5 (100)	3 (100)	3 (100)
Electron microscopy done in	16	22	4	0	1
Abnormal	6 (37)	10 (45)	2 (50)		1 (100)
Curvilinear inclusions	4	8	0		1
GRODS	1	2	2		
Curvilinear + GRODS	1	0	0		
Enzyme assay done	20	26	5	1	3
Low levels of enzyme	15	21	Normal	Normal	Normal
Pathogenic variant by genetic testing	4	5	4	3	3

Abbreviations: GRODS, granular round osmiophilic deposits; IPS, intermittent photic stimulation; PPR, photoparoxysmal response.

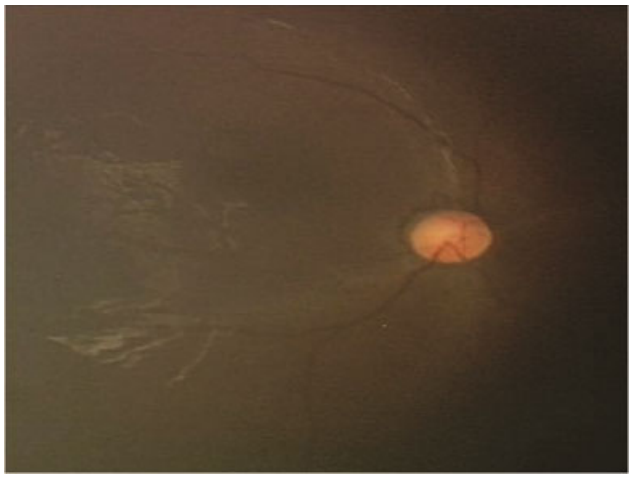

Fig. 1 Showing of fundus of CLN2 child with retinal arterial attenuation.

patients. ►**Fig. 1** shows retinal arterial attenuation in a child having CLN2.

Neuroimaging (MRI brain) revealed cerebral and cerebellar atrophy in all patients. It is noteworthy that two children with family history of similar complaints and early death whose initial brain MRI was normal, later developed cerebellar atrophy. ►**Fig. 2** shows MRI of the brain, with thalamic, cerebral, and cerebellar atrophy with periventricular white matter signal changes in a child with CLN1. EEG was abnormal in all children. EEG showed early photosensitivity in the form of photoparoxysmal response (PPR) at low intermittent photic stimulation (IPS) frequencies of 1 to 3 Hz in four children of CLN2 subtype.

Axillary skin biopsy done in 43 patients, revealed curvilinear inclusions in 13 (65%), five (18.51%) had granular round osmiophilic deposits (GRODS), one (5%) had both curvilinear and GRODS, and one (5%) had curvilinear plus fingerprint inclusions. The inclusions in different subtypes of NCL are GRODS seen in five cases of CLN1, curvilinear in 13 cases of CLN2, curvilinear and GROD in one case of CLN2, and curvilinear and fingerprint in one case of CLN3. ►**Fig. 3** shows Electron micrograph picture of curvilinear inclusions, membrane-bound fingerprint, and curvilinear profiles and GRODS.

One case each in CLN3, CLN5, and CLN11 were noted. All of them had seizures, regression, and ataxia. CLN3, a 5-year-old male child, had visual impairment and multifocal epilepsy on EEG. CLN5, 7-year-old male child with retinal artery attenuation and retinal pigmentary changes, generalized epileptiform discharges on EEG. CLN11, 14-year-old female child myoclonic jerks since the age of 13 year, and multifocal epileptiform discharges on EEG.

Enzyme study done in 46 patients had low enzyme levels in 36 (78%). While low TPP level was noted in 21, and low palmitoyl protein thioesterase (PPT) level was seen in 15. Genomic DNA from twenty-six patients analyzed by NGS showed pathogenic variant in 23 (►**Table 3**). The pathogenic variants identified include five each in TPP1 and CLN6, four in PPT1, three in each MFSD8 and CLN 8, one each in *CLN3, CLN5* and *GRN* gene. Six novel pathogenic variants noted, are mentioned in ►**Table 3**. Two children with *CLN2* mutation succumbed due to refractory epilepsy and aspiration pneumonia.

Three children of CLN2 with Epilepsia partialis continua (EPC) required intensive care treatment for three-week durations. One child with CLN2 was initially treated as autistic spectrum disorder. One child who presented initially with ataxia, subsequently with hyperammonemic encephalopathy with sodium valproate and treated as metabolic disorder was confirmed as CLN2. Encephalopathy improved with discontinuation of valproate. All NCL children required more than two anti-epileptic drugs to control seizures, except three cases in CLN1 group who were controlled well with sodium valproate alone. Antenatal diagnosis was offered for six families of which 4 families had affected fetus hence medical termination of pregnancy was advised.

Discussion

NCL is a rare autosomal recessive lysosomal storage disorder characterized by refractory seizures, progressive loss of vision and developmental delay or regression.[18] NCL is more common in males. In this study male constitutes 41 (68.33%) of cases, similar to that reported by other studies.[19,20]

CLN1 usually presents between 6-months to 2-years of age, however it also has late onset variants, such as late infantile, juvenile, and adult onset variants. The plausible reason for late onset presentation is compound heterozygotes for a typically "severe" mutation and a "mild" mutation. All children ($n = 21$) with infantile onset classified as CLN1 type had age at onset less than 2 years, except in two children who had onset after 2-years of age. However, we did not notice any compound heterozygous mutations in CLN1 gene.

The common presentation was developmental delay, ataxia, and regression of milestones, which was found in all patients in this study. The ophthalmological examination revealed attenuation of retinal vessels in 38.33%, retinal pigmentary changes in 18.33%, optic disc pallor in 18.33%, macular dystrophy in 15%, optic atrophy in 10%, and bull's eye maculopathy in 1.66%.

An important feature on MRI brain was cerebral and cerebellar atrophy seen in all 60 children. In addition, small thalamus, and cerebellar dentate nucleus hyperintensities were noted as reported in other studies.[21,22] Thalamic and caudate nucleus abnormalities are more common in CLN1 compared with CLN2. Autti et al emphasized that a decrease in T2 signal intensity in the thalami might be a sign of lysosomal storage disease.[23] It is noteworthy that two children with family history of similar complaints and early death whose initial brain MRI was normal later developed cerebellar atrophy.

EEG was abnormal in all. The most common abnormality seen in 72% was multifocal epilepsy with disorganised background. Characteristic finding of early photosensitivity in the form of PPR at low intermittent photic stimulation (IPS) frequencies of 1–3 Hz was seen in 4/25 (16%) children

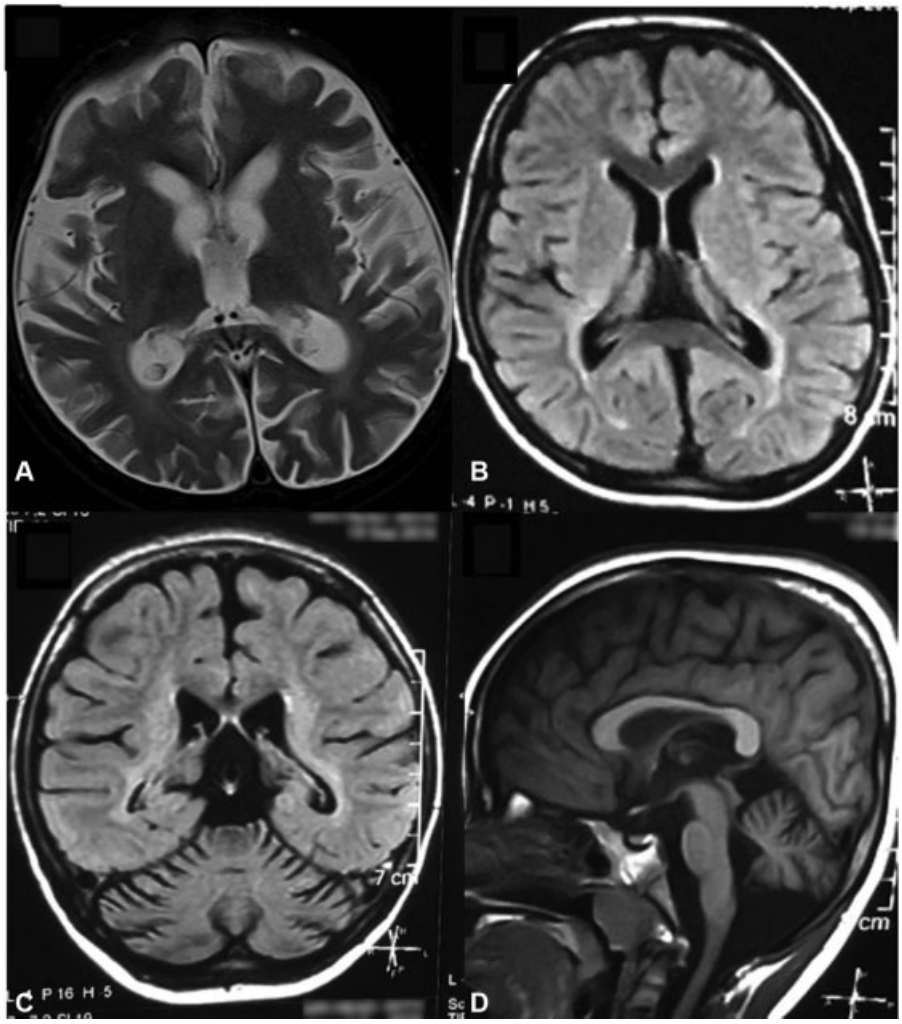

Fig. 2 Magnetic resonance imaging of brain showing. (**A**) T1W and (**B**) FLAIR thalamic and cerebral atrophy with signal changes in periventricular white matter and posterior limb of internal capsule. (**C, D**) Cerebellar atrophy in coronal and sagittal sections, respectively.

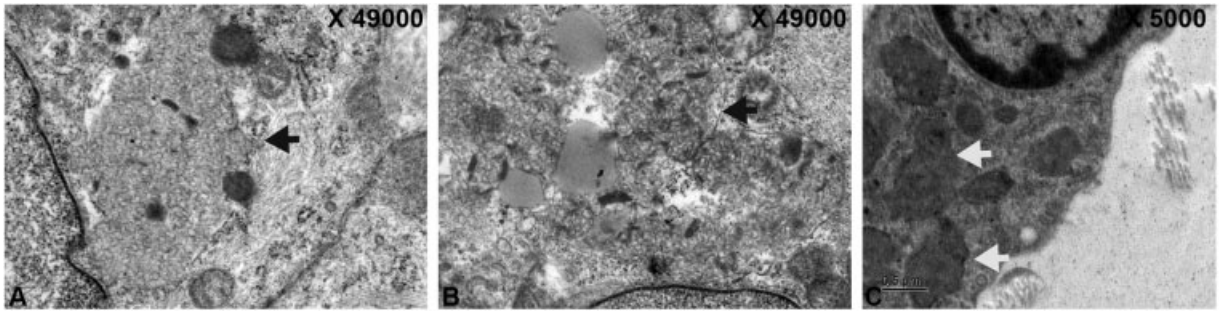

Fig. 3 Electronmicrograph showing. (**A**) Curvilinear Inclusions within the myoepithelium of the gland (black arrow). (**B**) Membrane bound fingerprint and curvilinear profiles (black arrow). (**C**) Granular round osmiophilic deposits (white arrows).

of CLN2 subtype. In a study using a standardized IPS method, Specchio et al,[24] reported PPR to be the hallmark feature of early CLN2 disease in most patients (93%). This finding contradicts with previous studies reporting that PPR is not a feature in all CLN2 patients[25,26] including the present study. The probable reason could be that these studies failed to use standardized EEG/IPS procedures and did not collect a comprehensive data, and likely that the phenomenon was present but not captured. When this occurs in a child presenting with any type of seizure, and particularly accompanied by delayed speech and/or ataxia, or MRI abnormalities (posterior white matter signal alteration or cerebellar atrophy) are present, a diagnosis of CLN2 should be considered. Ana Christina et al[20] showed that disorganized background activity was the earliest change in EEG in NCL in their study.

Skin biopsy done in 43 patients as part of diagnostic procedure subjected to ultrastructural study was abnormal

Table 3 Various pathogenic variants in different subtypes of neuronal ceroid lipofuscinosis

Patients (P)	Gene	Location	Variant	Zygosity	NCL Subtype	Classification (ACMG)
P1	*CLN3*	Exon 6	NM_001042432.2:c.388G > A (V130I p.Val130Ile)	HOM	NCL3	VUS
P2	*CLN5*	Exon3	ENST00000377453.3:c.634G > A (p.Ala163Thr)	HOM	NCL5	VUS
P3	*CLN6*	Exon 4	NM_017882.3:c.476C > T (p.Pro159Leu)	HOM	NCL6	VUS
P4		Intron 6	NM_017882.3:c.665 + 1G > T	HOM	NCL6	P
P5–P6		Exon 7	NM_017882.3:c.679G > A (p.Glu227Lys)	HOM	NCL6	LP
P7		Exon 7	NM_017882.3:c.775G > C (p.Gly259Arg)	HOM	NCL6	LP
P8–P9	*CLN8*	Exon 2	NM_018941.4:c.522C > G (p.Cys174Trp)	HOM	NCL8	LP
P10		Exon 3	NM_018941.4:c.592delA (p.Ile198PhefsTer10)	CH	NCL8	P
P10		Exon 3	NM_018941.4:c.610C > T (p.Arg204Cys)	CH	NCL8	P
P11	*GRN*	Exon 9	NM_002087.3:c.912G > A (p.Trp304Ter)	HOM	NCL11	P
P12	*MFSD8*	Intron 2	NM_152778.3:c.63-1G > A	HOM	NCL7	P
P13		Exon 5	NM_152778.3:c.268G > C (p.Ala90Pro)	HOM	NCL7	VUS
P14		Exon 10	NM_152778.3:c.894T > G (p.Tyr298Ter)	HOM	NCL7	P
P15–P18	*PPT1*	Exon 7	NM_000310.4:c.713C > T (p.Pro238Leu)	HOM	NCL1	LP
P19	*TPP1*	Exon 1	NM_000391.4:c.2T > C (p.Met1Thr)	HOM	NCL2	P
P20		Exon 8	NM_000391.4:c.1015C > T (p.Arg339Trp)	HOM	NCL2	P
P21–P22		Exon 9	NM_000391.4:c.1080C > A (p.Asp360Glu)	HOM	NCL2	LP
P23		Exon 12	NM_000391.4:c.1544T > G (p.Leu515Arg)	HOM	NCL2	LP

Abbreviations: ACMG, American College of Medical Genetics and Genomics; CH, compound heterozygous; HOM, homozygous; LP, likely pathogenic; NCL, neuronal ceroid lipofuscinosis; P, pathogenic; PPT, palmitoyl protein thioesterase; TPP, tripeptidyl peptidase; VUS, variant of unknown significance.

in 20 patients (46.52%). GRODS was noted in 25%, curvilinear deposits in 65%, combined curvilinear and fingerprint inclusions in one (5%) and curvilinear combined with GRODS in one patient (5%). In a study by Ana Christina et al,[20] it was seen that skin biopsies showed osmiophilic granular deposits with fingerprint profiles. Curvilinear inclusions are more common in CLN2. However, biopsy when positive suggests NCL but does not always differentiate between types of NCL and negative biopsy does not rule out disease.[27]

Epilepsy is less common in CLN1 as compared with CLN2. MRI shows thalamic atrophy in CLN1 in comparison to only cerebellar and cerebral atrophy in other subgroups. None of CLN1 cases showed PPR in LF PS in EEG. EM in this group showed GROD in six children. All children in CLN2 presented with refractory epilepsy. Three children had Epilepsia partialis continua. One child earlier was treated as a case of autistic spectrum disorders. Involuntary movements were more common in CLN2. EM showed curvilinear bodies (CL), in 8 children in CLN2. Above findings help in selecting cases to perform an enzyme assay before genetic testing. CLN3 usually presents with progressive visual loss followed by neurological involvement. EM shows presence of FP or FP/ CL/GROD.

Of the 46 patients subjected for enzymatic assay, 21 patients had low levels of the enzyme tripeptidyl peptidase (TPP), while 15 had low levels of PPT. Genetic analysis showed pathogenic variants in five each in *TPP1* and *CLN6*, four in *PPT1*, three each in *MFSD8* and *CLN 8*, and one each in *CLN3*, *CLN5*, and *GRN* gene. In this series, we reported six novel pathogenic variants. Kousi et al,[4] in their update, reported 365 NCL causing mutations in 14 genes. In an analysis of 34 patients from India, Sheth et al[28] reported 12 CLN1 and 22 CLN2 cases. There is complex correlation between phenotypes and genotype. Mutations in *CLN5*, *CLN6*, and *CLN8* can mimic *CLN2* phenotype. *CLN8* genotype can cause severe *CLN2* phenotype or mild progressive epilepsy with mental retardation.

We detected six novel pathogenic variants in this study. Pathogenic variant in *TPP1* gene at Ex12:c.1544T > G/p.L515R, presented with encephalopathy due to hyperammonamia following valproate administration. Second pathogenic mutation in *TPP1* gene at Ex3:c.1080C > A/p.Asp360Glu presented with autistic regression. Pathogenic variant in *CLN5* at Ex3: c.634G > A/p.Ala212Thr, had classical phenotype, is the first case of CLN5 reporting from India. Pathogenic variant in *MFSD8*,In2:c.63–1G > A/3′ splice site had small thalamus and periodic complexes in the EEG not been described previously. Pathogenic variant in *CLN6,* at In6:c.665 + 1G > G/5′split site and pathogenic variant in *CLN8* at Ex2:c.522C > G/p.Cys174Trp had classical phenotype.

In the present study, five children initially suspected to be having CLN2 were later turned out be CLN1 positives, following enzyme and genetic testing. Similarly, two children with initial clinical diagnosis of CLN1 turned out to be CLN2 after enzyme and genetic confirmation. One child in CLN2 in targeted exome sequencing did not reveal any pathogenic

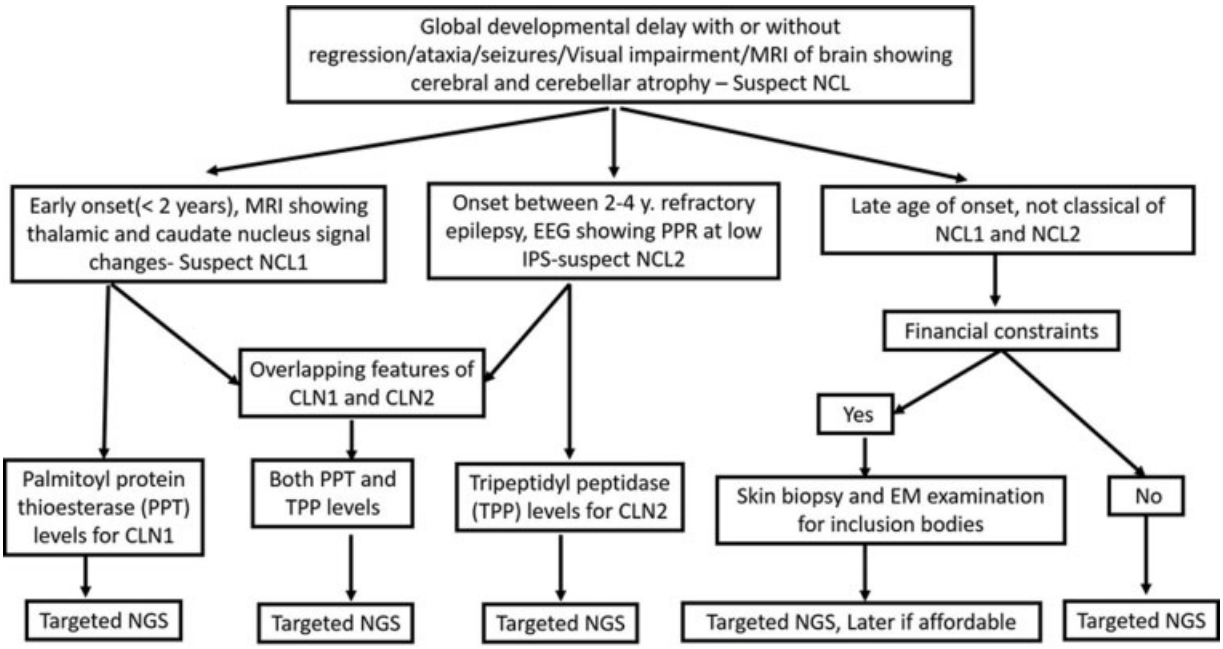

Fig. 4 Diagnostic algorithm for diagnosis of neuronal ceroid lipofuscinoses in resource poor setting.

variant. Electron microscopy done in 43 showed evidence of inclusions in twenty (46.52%). Targeted exome sequencing was done in 26. Pathogenic variant was noted in 23 (88.46%). Enzyme level was low in all children those confirmed CLN1 and CLN2 subtypes by genetic testing. Three children who could not be clinically subtyped nor enzyme assay performed was sent for NGS. The results are CLN2 in two and CLN1 in one child. Two children clinically suspected with CLN2 tested for TPP levels showed normal level; however, molecular testing revealed CLN8 subtype. Thus, a multimodal investigative approach played a role in the diagnosis of NCL.

EM though helpful in confirmation of diagnosis cannot be used as a tool for subtyping CLN. It is useful when diagnosis is not clear before considering enzyme analysis and genetic testing. In the present study enzyme analysis, in younger age group proved to be useful noninvasive technique for CLN1 and CLN2 genetic testing is better for prognostication and antenatal diagnosis. In older children, progressive course of myoclonic epilepsy is unclear; hence, EM can be considered for confirmation followed by targeted genetic testing for subtyping. We provided algorithm in ►**Fig. 4** for diagnosis of NCL in resource poor setting.

NCL is known to present with cognitive regression, refractory epilepsy, ataxia, visual impairment, cerebral and cerebellar atrophy, and photosensitivity to low-frequency photic stimulation in EEG. Our study added the following findings to the existing literature. NCL can present as developmental delay without regression. It can also present as autistic regression, EPC, and chorea-athetosis. MRI of brain can be normal in the initial stages of the disease. Multifocal epileptiform discharges are most common EEG abnormalities compared with photosensitivity to low frequency photic stimulation reported in the literature. We also noted periodic complexes in CLN8 subtype of NCL. CLN5 subtype is the first case reporting from India. We have also found six novel pathogenic variants of NCL.

To conclude, this was the largest cohort subtyping NCL in pediatric age group using multimodal investigative approach in a resource limited center. Our study identified six novel pathogenic variants. Diagnosis of NCL should be considered in children with global developmental delay with or without regression of milestones, involuntary movements, and refractory seizures. Thalamic and caudate nucleus changes with cerebellar atrophy on MRI brain in correlation with clinical features help in suspecting CLN1 compared with other subtypes. Refractory epilepsy and EEG showing PPR at low IPS frequencies of 1 to 3 Hz are suggestive of CLN2. Enzyme assay should be considered in clinically suspected CLN1 and CLN2 particularly in younger age group. Genetic studies are useful to subtype, for prognostication, and genetic counselling, especially for non CLN1 and CLN2 as there no biochemical tests available currently. Genetic testing is the key toward diagnosis of all cases of NCL, enzyme testing and EM are useful in the resource limited settings.

Authors' Contributions

V.K.G. dedicated in supervision, guidance, and reviewing the manuscript. H.V. and K.S. were involved in the management of the child and the preparation of the manuscript. V.M.S. supported in the collection of data and the preparation of the manuscript. G.N. and R.S. were involved in diagnosis and final manuscript preparation. M.B., S.B., and M.R.N. provided valuable inputs in the diagnosis and final manuscript preparation.

Conflict of Interest

None declared.

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