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Juvenile Batten disease (CLN3): Detailed Ocular Phenotype, Novel Observations, Delayed Diagnosis, Masquerades, and Prospects for Therapy.

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Running Title: Juvenile Batten Disease
Abstract (350 words)

**Purpose:** To characterize the retinal phenotype of juvenile neuronal ceroid lipofuscinosis (JNCL), highlight delayed and mistaken diagnosis, and propose an algorithm for early identification.

**Design:** Retrospective case series.

**Subjects:** Eight children (5 females) with JNCL.

**Methods:** Review of clinical notes, retinal imaging including fundus autofluorescence (FAF) and optical coherence tomography (OCT), electroretinography (ERG), and both microscopy and molecular genetic testing.

**Main Outcome Measurements:** Demographic data, signs and symptoms, visual acuity, FAF and OCT findings, ERG phenotype, and microscopy/molecular genetics.

**Results:**
Subjects presented with rapid bilateral vision loss over one to eighteen months, with mean visual acuity deteriorating from 0.44 LogMAR (range: 0.20 - 1.78 LogMAR) at baseline, to 1.34 LogMAR (0.30 LogMAR - light perception) at last follow-up. Age of onset ranged from 3 to 7 years (mean 5.3 years). The age at diagnosis of JNCL ranged from 7 to 10 years (mean 8.3 years). Six children displayed eccentric fixation, and six had cognitive or neurological signs at time of diagnosis (75%). Seven patients had bilateral bull’s-eye maculopathy at
presentation. Coats-like exudative vasculopathy, not previously reported in JNCL, was observed in one patient. OCT imaging revealed near complete loss of outer retinal layers, and marked atrophy of the nerve fibre and ganglion cell layers, at the central macula. An ‘electronegative’ ERG was present in four patients (50%), but with additional a-wave reduction; there was an undetectable ERG in the remaining four. Blood film microscopy revealed vacuolated lymphocytes and electron microscopy showed lysosomal (fingerprint) inclusions, in all eight patients.

Conclusions:

In a young child with bilateral rapidly progressive vision loss and macular disturbance, blood film microscopy to detect vacuolated lymphocytes is a rapid, readily accessible, and sensitive screening test for JNCL. Early suspicion of JNCL can be aided by detailed directed history and high-resolution retinal imaging, with subsequent targeted microscopy/genetic testing. Early diagnosis is critical to ensure appropriate management, counselling, support and social care for children and their families. Furthermore, although potential therapies for this group of disorders are in early phase clinical trial, realistic expectations are that successful intervention will be most effective when initiated at the earliest stage of disease.
Introduction

The neuronal ceroid lipofuscinoses (NCLs) are a group of inherited neurodegenerative lysosomal storage disorders that have been associated with 13 causative genes to date.\(^1\) Prevalence is 1 in 100,000 live births.\(^2\) Traditionally, the disease was divided into different forms dependent on the disease onset. Since disease onset and progression can vary substantially, genetic testing and confirmation of the underlying sequence variant is often required for a definite diagnosis. Consequently, a new gene-based nomenclature was introduced to facilitate disease classification.\(^3\) Classic CLN3 disease with juvenile disease onset, formerly known as juvenile neuronal ceroid lipofuscinosis (JNCL) and commonly referred to as Batten disease, is a form of NCL caused by sequence variants in the gene CLN3 (Ceroid Lipofuscinoscis, Neuronal, 3; OMIM: 204200). The gene codes for a transmembrane protein of unknown function.\(^4,5\) Presentation is typically in early childhood with vision loss at 4-10 years of age, behavioural and cognitive dysfunction (7-10 years), progressive motor decline and seizures (10-13 years), eventually leading to premature death in the second/third decade of life.\(^6,7\) The most common sequence variant in CLN3 is a homozygous 1kb deletion, accounting for approximately 85% of cases of JNCL.\(^8,9\) This deletion encompasses exons 7-8, resulting in a truncated, non-functional protein.\(^10\) Other variants in CLN3 can cause isolated adult onset retinal degeneration.\(^11,12\) Diagnosis of JNCL is confirmed by the presence of vacuolated lymphocytes and lysosomal (fingerprint) inclusions on blood film,\(^4,13-15\) alongside molecular genetic testing.\(^3\)

Visual impairment presents as the first symptom in over 80% of cases of JNCL at a mean age of around 5 years old.\(^16,17\) Retinal examination often shows a bulls-eye maculopathy, temporal optic disc pallor, peripheral retinal pigment epithelial disturbance (including bone spicule formation) and retinal vascular attenuation.\(^9,18-20\) In one study,
fundus imaging showed widespread atrophy of the retinal pigment epithelium (RPE) in 93% (n=24) of cases of confirmed CLN3 disease.\textsuperscript{21} However, because these retinal findings overlap with selected pathological hallmarks of more common disorders including retinitis pigmentosa, Stargardt disease and other inherited retinal diseases,\textsuperscript{22-24} the early diagnosis of JNCL often results in significant diagnostic challenge. Furthermore, one clinical study reported only two out of nine molecularly confirmed CLN3-JNCL patients as having bulls-eye maculopathy,\textsuperscript{25} and another suggested that only 20% of cases present with a bulls-eye appearance, further highlighting the difficulties in early detection of JNCL.\textsuperscript{21} Another less well recognised clinical feature which can be seen in JNCL is “eccentric vision” or “overlooking”, whereby the child will raise their eyes to overlook and fixate on a target object, and may be secondary to a relative degree of superior peripheral retinal sparing.\textsuperscript{26}

The electroretinogram (ERG) is valuable in the diagnostic armamentarium for JNCL,\textsuperscript{25} with marked ERG abnormalities invariably seen, including electronegative waveforms.\textsuperscript{22, 27, 28} As the disease progresses to more advanced stages the ERG shows significantly reduced cone responses and no recordable rod-specific responses.\textsuperscript{18} Cognitive and behavioural impairment, in particular mood, memory and attention (e.g. inability of the child to recall and accomplish three-step commands), usually appears approximately two years after the onset of visual decline, however these features may be present at first onset or occasionally in advance of visual symptoms; highlighting the importance of careful directed history in suspected cases.\textsuperscript{16, 17} Magnetic resonance imaging may show cerebral and cortical atrophy with demyelination.\textsuperscript{29}

Timely diagnosis of JNCL is often challenging. Given the rapidly progressive and unfavourable prognosis of the disease, early diagnosis is important both to provide timely
clinical management and support, and to also prepare for potential novel avenues of intervention. Herein, we describe eight cases of JNCL presenting at a single tertiary referral center in detail, highlighting delayed/mistaken diagnosis, diagnostic challenges, providing diagnostic insights, novel observations and recommendations, and also discuss the latest research avenues being explored and on-going/planned clinical trials.
Materials and Methods

Patient Identification

Patients with the diagnosis of JNCL and harboring likely disease-causing variants in \textit{CLN3} were identified from the Moorfields Eye Hospital Inherited Eye Disease database. Patients were included in this database after obtaining informed consent. This retrospective study adhered to the tenets of the Declaration of Helsinki and was approved by the Moorfields Eye Hospital ethics committee.

Assessment

Medical notes and clinical images were reviewed, including dilated fundoscopy, visual acuity (VA), electrophysiological testing (ERG), and retinal imaging including optical coherence tomography (OCT) and fundus autofluorescence (FAF).

The age of disease onset was defined as the age at which the first disease related symptom(s)/sign(s) were apparent. Screening for JNCL was done by microscopic evaluation of a peripheral blood film for the presence of vacuolated lymphocytes; followed by electron microscopy for storage (fingerprint) inclusions. Confirmation of the diagnosis was done by molecular genetic screening for \textit{CLN3} variants.

Methods of electrophysiological testing were adapted according to age and the ability of each individual to comply with testing. Full-field electroretinography (ERG) was performed to incorporate the International Society for Clinical Electrophysiology of Vision (ISCEV) standards, using a ganzfeld bowl and gold foil corneal electrodes (case 7) or lower eyelid skin electrodes (case 6). The ERGs in the other children were performed with skin electrodes without mydriasis, using flashes delivered by a Ganzfeld bowl (cases 3, 5, and 8).
or hand-held strobe (case 4), according to a modified protocol. ISCEV-standard pattern ERG (PERG), was performed using gold foil corneal (case 7) or skin electrodes. The mean subfoveal choroidal thickness was measured by enhanced depth imaging horizontal OCT crosshair scans (EDI-OCT, Heidelberg Engineering Inc., Heidelberg, Germany). Segmentation of macular ganglion cell layer (mGCL) thickness was obtained using the automated segmentation software for the Spectralis OCT device (Heidelberg Engineering, software version 1.10.2.0). For retinal thickness maps, three circular lines representing 1, 3 and 6 mm scan diameters (Early Treatment Diabetic Retinopathy Study; ETDRS macula) were obtained. The macular scans were performed in the 30° perifoveal area using a 30°×25° OCT volume scan. The average of all points within the inner 1 mm diameter circle was defined as the central subfield thickness. The intermediate 3mm ring was divided into the inner superior, inner nasal, inner inferior and inner temporal subfields and average values were calculated per sector in each eye.

Results

Clinical Findings

All eight ascertained patients were first seen at Moorfields Eye hospital over a period of 8 years (2009-2017) and received the diagnosis of CLN3-disease in 6-18 weeks after their first visit (mean: 10.7 weeks). They were referred with poor visual acuity, with all having experienced a period of rapid visual decline before their referral to our tertiary center, ranging from one to eighteen months in duration. Mean visual acuity (±SD, range) at disease onset was 0.44 LogMAR (±0.44, 0.20-1.78 LogMAR). Mean visual acuity (±SD, range) by the time of diagnosis was 1.34 LogMAR (±0.61, 0.30 LogMAR - light perception). The age of disease onset ranged from 3 to 7 years (mean age 5.3 years). The time from disease onset to
diagnosis ranged from 1.5 to 5 years (mean time 2.9 years). Age at diagnosis of JNCL ranged
from 7 to 10 years (mean age 8.3 years). The medical history prior to first presentation in
five children was unremarkable (n=5, 62.5%), one patient had speech delay and learning
difficulties, and a further patient had been hospitalised aged 8 weeks with hypoglycaemia
and low cortisol. Table 1 summarizes the clinical findings.

Six out of eight children were seen by an ophthalmologist at their local hospital prior
to referral to our tertiary center (n=6, 75%). The other two cases (cases 6 and 7) were
referrals from an orthoptist and General Practitioner respectively. In three cases (n=3,
37.5%, Cases 2, 5 and 7) legal guardians had reported concerns about vision from as early as
3 years of age (range 3 to 7). In two cases (Cases 2 and 4), teachers had reported visual
disturbance. At the time of referral, the presumed diagnoses in the eight cases were
Stargardt disease (n=1), severe retinal dystrophy (n=3), and unexplained visual loss (n=4)
(Table 1).

Of note, 6 out of 8 patients had eccentric fixation/'overlooking' (75%) either on
observation or on directed history. On directed detailed history, 6 out of 8 patients had
cognitive or neurological signs (75%) - including change in mood, behaviour, balance, or
memory. MRI was carried out in three children and was unremarkable.

Retinal Imaging

As shown in Figures 1 and 3, all but one patient (case 7) presented with a bull’s-eye
maculopathy. Optic disc pallor, arteriolar attenuation, and subtle granularity of the RPE was
observed in all cases. Case 4 also developed inferior peripheral exudation, in keeping with a
Coats-like vasculopathy (not previously reported in JNCL), which spontaneously improved
over 12 months (Figures 1 and 3); and a mild mid-peripheral pigmentary retinopathy with bone spicule formation.

Macular FAF images (Figure 2) depict marked foveal hypoautofluorescence with varying degrees of surrounding diffuse reduction in macular autofluorescence in all patients (n=8, 100%). In addition, a perifoveal ring of increased autofluorescence was present in cases 3 and 6. Peripheral autofluorescence was variably decreased among the patients; ranging from mildly diffuse hypoautofluorescence (cases 3, 5 and 6), to markedly diffuse hypoautofluorescence (case 4). Case 1, 2, 7, and 8 show variable extend of decrease autofluorescence between the two aforementioned groups. In cases 1 and 7, mild RPE mottling was seen in the periphery (Figure 2). In case 4, striae of decreased signal were observed in the periphery and perifoveal area (Figure 2).

OCT was available for analysis in 7 cases. In all cases OCT imaging revealed near complete loss of photoreceptor cells, atrophy of the outer nuclear layer, outer plexiform layer, and marked atrophy of the nerve fibre and ganglion cell layers (Figure 3). The ellipsoid zone was markedly disrupted/absent, and it was difficult to identify remnants of the photoreceptor layer, due to debris. Hyper-reflective dots were visible at the level of the expected photoreceptor layer (Figure 3).

Mean subfoveal choroidal thickness was within age-adjusted normal limits, (mean 336 µm right eye and 330 µm left eye). Automated segmentation of the mGCL was performed in four patients (Cases 1, 3, 4 and 6). The values obtained from the 1 mm diameter central subfield area were excluded from analysis as the mGCL in the central subfield was very thin, precluding adequate segmentation. The values corresponding to the 6mm outer ring were excluded as they fell outside the scanning area due to eccentric
fixation. The average mGCL of the intermediate 3mm ring was 10.84 µm (SD: ±2.87 µm) 
(Supplementary Figure 1). The average mGCL thickness was 44.1 µm ± 9.22 µm, with mean 
(5th-95th percentile) normative value for children 5-17 years (n=276) being 51.6 µm (44.43-
58.25 µm).  

In addition to mGCL thinning, changes at the level of nerve fiber layer (NFL) and 
internal limiting membrane (ILM) were observed in all patients. Radial retinal striae were 
observed within the vascular arcades in five cases (n=5, 62.5%, Figures 3 and 4). Striae 
(folds) resembling epiretinal membranes, but without vessel alterations, were seen on 
fundoscopy and color fundus photography (Figure 4 - Case 2). No definite membrane was 
seen joining the tips of the folds on OCT (Figure 4, Cases 1, 5, 7 and 8). In contrast, gliosis of 
the inner retina, presented as increased reflectivity at the ILM,  was evident in all but one 
patient (case 3). An increased, patchy (linear) signal observed at the level of the ILM with 
severe disruption of the NFL, appears to have led to more prominent “folding” nasally, 
possibly related to greater NFL thickness (Figure 3). Foci of increased signal, instead of 
patchy linear areas, were observed in the three cases without folds (Figure 4, Cases 3, 4 and 
6). The external limiting membrane (ELM) was present, however disrupted (Figure 3), and 
no folds were present in these patients, perhaps representing an earlier stage of the 
disease. The mean age at the time of OCT imaging was 8.9 years for patients with striae and 
8.0 years for patients without striae. 

Electrophysiological Assessment 

Full-field and flash ERGs were recorded in all patients under photopic and scotopic 
conditions. Cases 1, 2, 4 and 5 had undetectable ERGs, in keeping with severe rod and cone 
photoreceptor dysfunction (Figure 5A). Cases 3, 6, 7 and 8 had undetectable scotopic dim
flash ERGs; strong flash ERGs were electronegative but with additional a-wave reduction. The photopic single flash ERGs had a low b:a ratio but with additional a-wave reduction in cases 7 and 8. The LA 30Hz flicker ERGs were mildly delayed in all cases with a detectable response (cases 3, 6, 7 and 8) (Figure 5A and 5B). The findings were consistent with marked generalised inner retinal dysfunction of rod (cases 3, 6, 7 and 8) and cone (cases 7 and 8) systems, with additional rod and cone photoreceptor involvement in all cases. Pattern ERGs were undetectable in the six cases tested in keeping with severe macular dysfunction.

Blood film Microscopy/ Electron Microscopy

Blood film microscopy performed for all eight patients demonstrated vacuolated lymphocytes. Electron microscopy was done sequentially in seven patients and all showed lysosomal (fingerprint) inclusions.

Molecular Genetics

All patients were molecularly confirmed as harboring likely disease-causing variants in CLN3. Six out of eight patients were homozygous for the common 1.02kb deletion. Case 8 was homozygous for c.(962+dup), case 7 was compound heterozygotes for c.(1056+3A>C) and deletion of exon 2-5. One patient (case 3) was referred initially with 'molecularly confirmed' Stargardt disease for consideration of clinical trials/studies. The clinical presentation/detailed history/imaging was not in keeping with Stargardt disease and so investigation was initiated for JNCL. The previously identified compound heterozygous ABCA4 variants were further assessed in silico, with one of the variants determined to be
unlikely to be pathogenic. Determination of disease-causation of ABCA4 variants is highly challenging given the vast allelic heterogeneity and highly polymorphic nature of this large gene.
Discussion

This report characterizes the early retinal phenotype of juvenile Batten disease, highlights the importance of early diagnosis of CLN3 disease in young children who present with rapid visual loss, with or without the presence of neurological or cognitive symptoms, and describes conditions that can masquerade as CLN3 disease.

Our case series identified a significant delay in diagnosis in all 8 children, with an average delay of 2.9 years from first presentation to diagnosis, in line with previous studies reporting a delay of 1.3 to 4 years.\textsuperscript{9} It is of note that in the past, the diagnosis was often only made after the onset of seizures despite prior visual failure,\textsuperscript{26} whereas, early diagnosis should now be possible following the advancements in retinal imaging and molecular testing that are now readily available.

There are several clinical symptoms (likely to require a directed careful history – including eccentric viewing and changes in mood/behaviour/cognition/memory), and signs on examination/detailed imaging, that should warrant directed investigations to promptly diagnose CLN3-JNCL. These include visual loss, which is characteristically rapid, and was present in all of our cases; most commonly reported in the literature between 6 to 8 years of age.\textsuperscript{9, 11, 25, 28} Other associated behavioural and cognitive impairments were also present in six out of eight cases, however these were often not identified at the time of visual complaints and / or not investigated or considered pertinent to the unexplained/otherwise explained visual loss – thereby further contributing to delayed diagnosis.

Fundus abnormalities seen in CLN3 disease such as “bulls-eye” maculopathy, retinal vascular attenuation, and optic disc pallor were present in our series, however, these are also features of other severe retinopathies.\textsuperscript{23, 24} Previously, eccentric fixation or “overlooking” has been attributed to a degree of superior peripheral retinal sparing.\textsuperscript{26}
Despite the majority of the patients in our study having eccentric fixation/overlooking (Table 1), the disease appeared relatively symmetrical between the superior and inferior retina on fundus autofluorescence imaging (Figure 3); suggesting no obvious anatomical difference and also no functional difference (indirectly) – although, we cannot exclude that direct functional testing may identify a difference between superior and inferior retinal sensitivity.

Abnormalities in OCT features can be very helpful in guiding the clinician to directly investigate CLN3-disease; including the profound degree and extent of outer retinal loss of lamination at a relatively young age, significant inner retinal thinning, and also the presence of increased inner retinal reflectivity. The increased reflectivity has been described as being secondary to epiretinal membrane (ERM) formation in several reports. Haisworth et al, identified ERM in 33% of their cohort (n=24), based on fundus appearance alone.21 More recently, Dulz et al described a striation pattern without ERM in all their patients (n=11, mean age 14.4 years), using OCT.9 In our cohort, all patients had reflectivity changes in the nerve fibre layer (NFL) and ILM. Although the mean age of our cohort was lower (average age 8.9 yrs), retinal striation was observed in 62.5% of the patients – distinct from typical ERM; with the three patients without striae being on average a year younger (8 years) (Figure 4). Our findings of profound diffuse macular ganglion cell thinning are in keeping with the degenerative NFL and mGCL loss reported in histological studies.35, 36

The scotopic ERGs in four of four cases with a detectable response had electronegative waveforms, consistent with dysfunction that is post-phototransduction or inner retinal, but with a-wave reduction indicating significant additional loss of photoreceptor function. An electronegative ERG has often been associated with juvenile \( CLN3 \) disease and may prompt screening in some cases, particularly if the photopic ERG
shows a reduced b:a ratio.\textsuperscript{25} It is noted however that an electronegative ERG is not
diagnostic and is a feature of congenital stationary night blindness, X-linked retinoschisis
and many other disorders.\textsuperscript{22, 37-39}

One patient was referred initially with 'molecularly confirmed' Stargardt disease
(STGD) for consideration of clinical trials/studies. The clinical presentation/detailed
history/imaging was not entirely typical of STGD and so investigation was initiated for JNCL.
The previously identified compound heterozygous \textit{ABCA4} variants were further assessed \textit{in}
silico, with one of the variants determined to be unlikely to be pathogenic. Determination of
disease-causation of \textit{ABCA4} variants is highly challenging given the vast allelic heterogeneity
and highly polymorphic nature of this large gene. This case highlights (i) that the clinician
needs to be mindful that severe \textit{ABCA4}-retinopathy associated with generalised cone-rod
dystrophy at an early age can masquerade as \textit{CLN3}-JNCL, (ii) the difficulties in definitively
ascribing disease-causation to identified sequence variants in this era of genomic
ophthalmology and more readily-accessible genetic testing, and (iii) further illustrates the
challenges in diagnosing \textit{CLN3} disease in a timely fashion and the potential consequences of
mistaken diagnosis.

Early diagnosis of JNCL remains a diagnostic challenge, particularly as other severe
retinal dystrophies can present with early onset visual loss. Moreover, associated non-
ocular symptoms/signs are often compartmentalised and investigated separately which can
lead to further delay. We suggest that a child with bilateral rapidly progressive vision loss,
with or without cognitive/behavioural problems at presentation, should have microscopy of
a peripheral blood film to detect the presence of vacuolated lymphocytes, which can act as
a sensitive screening test (all patients with \textit{CLN3} disease will test positive); followed by
electron microscopy for storage (fingerprint) inclusions.\textsuperscript{15} Diagnostic confirmation should be
done with molecular genetic screening of CLN3 (Figure 6).

The most common variant in CLN3-JNCL is a 1kb deletion resulting in a frameshift
and a truncated protein product.\textsuperscript{10} In our cohort, 75\% of cases were homozygous for this
deletion and had similar clinical presentations. Case 8, who harbored the c.(962+dup)
variant homozygously was reported to have better VA at presentation, but by the time of
diagnosis had similar VA to the other patients. Case 7, the only compound heterozygote in
our cohort (c.(1056+3A>C) and deletion of exon 2-5) had a milder ocular phenotype, with
the most preserved VA in the cohort and a degree of residual ellipsoid zone on OCT (Figure
3). As this patient presented with early cognitive and behavioural abnormalities, it could be
speculated that this genetic variant may have less deleterious effects on vision. Case 7 also
had electronegative ERGs but with an altered waveform morphology of the rod ERG, which
was not observed in any of the other subjects. The significance of this ERG finding is
uncertain. Although there is no treatment that has yet been shown in a clinical trial to
benefit patients with JNCL, it is important to explain the genetics of the disease to the
parents, provide genetic counselling, offer to follow the child yearly for routine eye care,
and offer to refer them to a pediatric neurologist, knowledgeable pediatrician, or family
practitioner who is willing and able to help follow and care for the child. This includes
specialist knowledge of certain medications that are more likely to induce adverse side
effects when given to a child with JNCL. Referral to international foundations that support
research on the NCL disorders, social workers, or local or national support groups of parents
who have children with JNCL may help parents and families cope with issues commonly
seen as the disease progresses.
To date, there are no treatments available for juvenile CLN3 Batten disease or other forms of NCL. The majority of studies has focused on developing therapeutic interventions to combat the neurodegeneration in NCL, including enzyme replacement therapy, gene therapy, stem cell transplantation and pharmacological approaches. Most notably, CLN2 disease patients that received biweekly intraventricular infusion of soluble CLN2 enzyme (NCT01907087, NCT02485899) showed no significant decline in motor or language skills and overall disease progression was considerably slowed during the reporting period. The treatment has now received FDA and EMA approval. A phase I/II trial has also started for CLN6 disease using gene therapy administered by a single intrathecal injection of adeno-associated virus (AAV) 2/9 carrying CLN6 (NCT02725580). This study is on-going, but data from 8 out of 12 patients two years post vector injection are available and show promising preliminary results. Based on these data, a phase I/II clinical trial has started recruiting for CLN3 disease, to investigate intrathecally administered AAV2/9-CLN3 (NCT037770572). Although, these studies will primarily assess treatment safety and effects on neurological features, they may also help to determine whether brain-directed gene therapy has any impact on vision in CLN6 and CLN3 patients. As CLN6 and CLN3 encode membrane-bound proteins that are not passed on to neighbouring cells, it is more likely that gene therapy directly targeting the eye will be more effective to prevent retinal degeneration in both diseases. A proof-of-concept study demonstrated that ocular gene therapy is therapeutic in Cln6nclf mice, a mouse model for CLN6 disease, when the inner retina was treated. Preclinical ocular gene therapy for CLN3 disease has not been described yet. However, a similar gene therapy approach targeting the cells of the inner retina as used in Cln6nclf mice could also be effective in Cln3-deficient mice, and may also be relevant to human CLN3 disease (both syndromic and non-syndromic).
Herein, we have described cases of juvenile CLN3 disease in detail, highlighting delayed/mistaken diagnosis, diagnostic challenges, providing diagnostic insights, novel observations and recommendations, and also highlighting the latest clinical research and on-going/planned clinical trials. We have also emphasized the role of the ophthalmologist, and paediatrician or primary care provider and the need for additional continued support for the family. Whilst timely diagnosis of JNCL is often challenging, given the rapidly progressive and unfavourable prognosis of the disease, early diagnosis is important both to provide timely clinical management and support, and to facilitate access to novel therapeutic interventions at the early disease stages.
LEGENDS

Figure 1. Clinical features on color fundus photography.
Color fundus photographs of five cases with juvenile neuronal ceroid lipofuscinosis; depicting optic disc pallor, macular atrophy with subtle granularity of the retinal pigment epithelium (RPE) and retinal arteriolar attenuation. Note the pigmentary changes reminiscent of bone spicules and unilateral Coats-like reaction in case 4. The second row for case 4 shows the exudation at baseline and its improvement over a follow-up period of 12 months.

Figure 2. Fundus autofluorescence findings.
Fundus autofluorescence images showing marked foveal hypoautofluorescence with varying degrees of surrounding diffuse reduction in macular autofluorescence. Cases 3 and 6: A ring of increased autofluorescence (white arrow heads). Cases 3, 5 and 6 show mild diffuse peripheral hypoautofluorescence and, case 4 shows advanced diffuse hypoautofluorescence. Case 1, 2, 7, and 8 show variable extend of decrease autofluorescence between the two aforementioned groups.

Figure 3. Optical coherence tomography findings.
Spectral-domain optical coherence tomography (SD-OCT) macular scans for all patients in the cohort, at the time of diagnosis, depicting significant macular atrophy with almost complete loss of the ellipsoid zone, hyper-reflective dots at the outer retinal level, marked atrophy of the outer nuclear layer, outer plexiform layer, ganglion cell layer and nerve fibre layer. Glial fibrosis is observed at the level of the inner retina. The white arrow heads mark possible areas of residual ellipsoid zone. The orange arrow heads mark an a example of
continuous, even though altered, external limiting membrane, despite the excessive loss of the photoreceptor layer. The white borders delineate regions of interest shown in greater magnification in Figure 4.

Figure 4: Macular striation and degenerative changes

Striation and/or degenerative changes were present in all patients. High magnification of the marked areas in Figure 3 are shown, from horizontal OCT scans of the nasal fovea. Retinal radial striae within the vascular arcades were observed in cases 1, 5, 7 and 8. Striae, resembled the appearance of epiretinal membranes on fundoscopy and color fundus photography, but no vessel alterations are seen and no definite membrane observed joining the tips, marked with white arrow heads, of the folds seen on OCT. Foci of increased signal, marked with yellow arrow heads, were observed in Case 3, 4 and 6 who did not have folds. In contrast to case with folds, where the areas of increase signal were greater in size and had a more linear distribution.

Figure 5: Electroretinography

Electroretinography recorded with lower eyelid skin electrodes in cases 4, 5, 6 and 8 (A) and with corneal electrodes in case 7 (B). Note 20ms pre-stimulus delay in single flash ERGs. Electrode-specific control recordings are shown for comparison but without a 20ms pre-stimulus delay in B. ISCEV-standard stimuli were used in case 4 (without mydriasis) and in cases 6 and 7; a strobe was used to deliver flashes in subjects unable to comply with Ganzfeld testing (dim flash rod ERG/DA0.01 ERG excluded from the protocol). ISCEV
standard testing (cases 6 and 7) included the dark-adapted (DA) ERGs (flash strengths 0.01 and 10.0 cd.s/m²; DA 0.01 and DA 10.0) and light-adapted (LA) ERGs for a flash strength of 3.0 cd.s/m² (LA 3.0; 30Hz and 2Hz). Data are shown for one eye but all had symmetrical responses. Broken lines replace blink/eye movement artefacts occurring after ERG b-waves for clarity. Recordings from patients are superimposed to demonstrate reproducibility. Note small differences in scaling and format of skin ERGs (A) related to use of different recording equipment. See text for ERG analysis.

Figure 6: Diagnostic algorithm for juvenile neuronal ceroid lipofuscinosis (JNCL), CLN3-associated disease.

In a child with bilateral rapidly progressive vision loss, microscopy of peripheral blood film can detect the presence of vacuolated lymphocytes, a sensitive screening test for JNCL, followed by electron microscopy for lysosomal storage inclusions. Confirmation of the diagnosis should follow with molecular genetic testing for CLN3 variants.

FOOTNOTES

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| Sex (F/M) | Age at onset (y) | Initial clinical findings at first evaluation | VA; R, L (LogMAR) | Neurological/behavioural signs | Rapid visual decline within: | Diagnosis on referral to MEH | Age at Diagnosis (y) | Clinical features at time of diagnosis | VA; R, L (LogMAR) | Eccentric fixation/‘overlooking’ | Neurological/behavioural signs |
|----------|-----------------|----------------------------------|-------------------|-----------------------------|-----------------------------|----------------------------|-----------------|--------------------------------|-------------------|--------------------------------|-----------------------------|
| Case 1   | F               | 5                               | Macular atrophy, retinal degeneration on OCT | 1.78, 1.78 | Speech delay, ‘clumsiness’, seizures | 6 months | Severe retinal dystrophy | 10              | Profound macular atrophy, optic disc pallor, retinal vascular attenuation | PL, PL | ✔ | Speech delay, clumsiness |
| Case 2   | F               | 5.5                             | Nystagmus, reduced vision, poor night vision | 0.60, 0.48 | None | 1 month | Severe retinal dystrophy | 7               | Rotary nystagmus, pale optic discs, bull’s-eye maculopathy, bilateral epiretinal membrane, retinal vascular attenuation | ✔ | ✔ | None |
| Case 3   | F               | 3                               | Foveal thinning, optic disc pallor, bull’s-eye maculopathy, poor color vision/night vision | 0.35, 0.80 | Behavioural and cognitive decline (ASD?); ‘clumsiness’ | 12-18 months | Molecularly confirmed Stargardt disease (ABCA4) | 8               | Profound loss of inner and outer retina, bilateral epiretinal membrane, bull’s-eye maculopathy | 1.35, 1.60 | ✔ | ✔ |
| Case 4   | M               | 7                               | Bilateral macular changes, optic disc pallor | 0.60, 0.75 | Emotional difficulties, cognitive decline | 1 year | Severe retinal dystrophy | 9               | Pale optic discs, attenuated vessels, bilateral macular atrophy | 1.20, 1.30 | ✔ | Behavioural and cognitive decline, clumsiness |
| Case 5   | F               | 4                               | Visual impairment | 0.80, 0.80 | None | 12-18 months | Unexplained vision loss | 9               | Bilateral macular atrophy | 1.0, 1.10 | NR | |
| Case 6   | M               | 6                               | Non correctable vision, poor color vision | 0.70, 0.50 | None | 1 year | Unexplained vision loss | 7               | Loss of central retinal structure, bilateral epiretinal membrane, poor color /night vision, pale optic discs | 1.30, 1.23 | ✔ | |
| Case 7   | M               | 6                               | Unexplained poor vision | 0.26, 0.20 | None | 1 year | Unexplained vision loss | 8               | Bilateral epiretinal membrane, outer retinal loss, pale optic discs | 0.50, 0.30 | ✔ | |
| Case 8   | F               | 6                               | Esotropia, left amblyopia | 0.18, 0.48 | Behavioural decline | 1 year | Unexplained vision loss | 8               | Bilateral macular atrophy, foveal sheen | 1.30, 1.30 | ✔ | |

ASD=Autistic spectrum disorder; NR = Not recorded; PL=Perception of light, R; Right Eye, L; Left Eye
Ophthalmology
- Child with rapidly progressive vision loss
- Cognitive/Behavioral/Mood Changes
- Retinal Imaging
- Electronegative ERG

Pathology
- Peripheral Blood Film Microscopy
- Vacuolated Lymphocytes
- Electron Microscopy
- Storage Lysosomal Inclusions

Genetic testing
- 1kb deletion CLN3
- Other CLN3 Variants
Précis (Highlights)

This report highlights the importance of considering juvenile neuronal ceroid lipofuscinosis disease in young children who present with rapid visual loss, with or without the presence of neurological or cognitive symptoms.