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## Diagnosis of neuronal ceroid lipofuscinosis type 2 (CLN2 disease): Expert recommendations for early detection and laboratory diagnosis



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## ABSTRACT

Neuronal ceroid lipofuscinoses (NCLs) are a heterogeneous group of lysosomal storage disorders. NCLs include the rare autosomal recessive neurodegenerative disorder neuronal ceroid lipofuscinosis type 2 (CLN2) disease, caused by mutations in the tripeptidyl peptidase 1 (*TPP1*)/*CLN2* gene and the resulting TPP1 enzyme deficiency. CLN2 disease most commonly presents with seizures and/or ataxia in the late-infantile period (ages 2–4), often in combination with a history of language delay, followed by progressive childhood dementia, motor and visual deterioration, and early death. Atypical phenotypes are characterized by later onset and, in some instances, longer life expectancies. Early diagnosis is important to optimize clinical care and improve outcomes; however, currently, delays in diagnosis are common due to low disease awareness, nonspecific clinical presentation, and limited access to diagnostic testing in some regions. In May 2015, international experts met to recommend best laboratory practices for early diagnosis of CLN2 disease. When clinical signs suggest an NCL, TPP1 enzyme activity should be among the first tests performed (together with the palmitoyl-protein thioesterase enzyme activity assay to rule out CLN1 disease). However, reaching an initial suspicion of an NCL or CLN2 disease can be challenging; thus, use of an epilepsy gene panel for investigation of unexplained seizures in the late-infantile/childhood ages is encouraged. To confirm clinical suspicion of CLN2 disease, the recommended gold standard for laboratory diagnosis is demonstration of deficient TPP1 enzyme activity (in leukocytes, fibroblasts, or dried blood spots) and the identification of causative mutations in each allele of the *TPP1*/*CLN2* gene. When it is not possible to perform both analyses, either demonstration of a) deficient TPP1 enzyme activity in leukocytes or fibroblasts, or b) detection of two pathogenic mutations in *trans* is diagnostic for CLN2 disease.

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## 1. Introduction

Neuronal ceroid lipofuscinosis type 2 (CLN2) disease (OMIM 204500) is a rare autosomal recessive lysosomal storage disorder that results from deficient activity of the lysosomal exopeptidase tripeptidyl peptidase 1 (TPP1) enzyme (EC 3.4.14.9) caused by mutations in the

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TPP1/CLN2 gene (GenBank accession no. NM\_000391.3) [1]. As in other neuronal ceroid lipofuscinosis (NCL) disorders (Table 1), CLN2 disease leads to intralysosomal accumulation of autofluorescent storage materials and neuronal loss.

The *in vivo* substrate(s) of TPP1 are not known, and the molecular pathology of TPP1 enzyme deficiency is poorly understood [2]. CLN2 disease incidence estimates range from 0.22 to 9.0 per 100,000 live births, but it is possible that it is under-recognized [2–4].

The classic phenotype of CLN2 disease is the most common form of NCL with late-infantile onset (roughly defined as ages 2–4 years) and generally manifests with new-onset seizures and/or ataxia, typically in combination with a history of early language delay [5]. Classic phenotype disease progression is rapid, leading to the loss of acquired developmental milestones, new or worsening ataxia, movement disorders (myoclonus, dystonia, and chorea), progressive dementia, and eventual loss of vision [3,5–9]. A majority of those diagnosed with CLN2 disease die prematurely after becoming bedridden and blind. The more rare atypical phenotypes are characterized by varied ages of initial presentation and/or longer life expectancy [5,10–12]. An example of atypical CLN2 disease is spinocerebellar ataxia autosomal recessive 7 (SCAR7; OMIM 609270), which was initially described as a distinct disorder but is in fact caused by deficient TPP1 enzyme activity; individuals with SCAR7 develop ataxia and cerebellar atrophy but do not develop seizures or loss of vision [12].

In May 2015, 13 international laboratory and clinical NCL experts met both to develop a CLN2 disease-specific diagnostic algorithm (Fig. 1) and to define the gold standard diagnostic laboratory tests to support an early and accurate diagnosis of CLN2 disease.

## 2. Clinical suspicion and paths to diagnosis of CLN2 disease

Developing a specific suspicion of CLN2 disease through differential diagnosis is often a protracted process, due in part to lack of pathognomonic signs at onset. Based on the expert meeting discussions, three general paths lead to a laboratory diagnosis of CLN2 disease, depending on degree of CLN2 disease suspicion (Fig. 1).

### 2.1. High suspicion of CLN2 disease

CLN2 disease is rarely suspected or diagnosed at initial presentation to the clinic unless there is already a known, affected family member. Because CLN2 disease is one of the most common of NCL disorders [13], children who ultimately present to an NCL specialist with ataxia, worsening unprovoked seizures, history of language delay, and/or are at a developmental stand-still will follow a clear, direct path specific for a high suspicion of classic CLN2 disease (Fig. 1, center) [5]. Typically, diagnosis only occurs after a series of misdiagnoses, as the early disease course is clinically similar to many other seizure and/or metabolic disorders.

An EEG is often the first clinical test performed irrespective of the level of specific clinical suspicion (Fig. 1). In cases of CLN2 disease, electroencephalograms (EEGs) may detect irregular activity, a slowing of background activity, and epileptiform abnormalities in posterior regions. Visual evoked potentials reveal an increase of latency; optical coherence tomography may detect ocular abnormalities; and electroretinograms may be diminished [3,5,8,14,15]. Ocular abnormalities and vision defects can be subtle initially but increase in prominence with disease progression [5,8,15].

**Table 1**

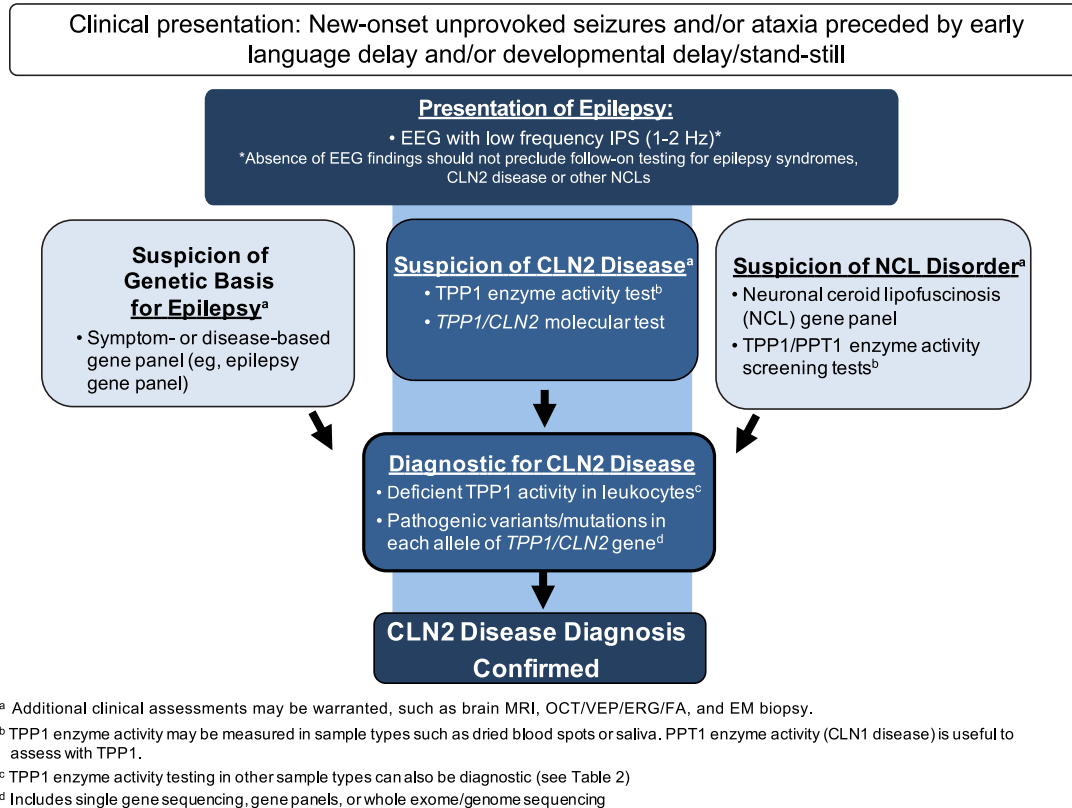
The neuronal ceroid lipofuscinosis disorders.

Adapted from the DEM-CHILD algorithm, <http://www.dem-child.eu/index.php/ncl-diagnostic-algorithm.html>, and Schulz et al. 2013 [14].

Age and clinical presentation	Genes	Protein products, in bold if enzyme assay widely available	Classical clinical presentation by age group
Newborns (through infantile)	• Epilepsy	<b>PPT1</b> (lysosomal lipid hydrolase)	Classical
	• Microcephaly	<b>CtsD</b> (lysosomal peptidase)	Classical
		KCTD7 (unknown function; homology to potassium channel tetramerization domain)	Classical
Young children (late infantile)	• Developmental delay or regression	<b>PPT1</b> (lysosomal lipid hydrolase)	Classical
	• Newly occurring epilepsy of unknown cause	<b>TPP1</b> (lysosomal exopeptidase)	
		(Soluble lysosomal protein)	
		(Transmembrane protein)	
		MFSD8 (transmembrane protein)	
		(Transmembrane protein)	
		<b>CtsD</b> (lysosomal peptidase)	
School-aged children (juvenile)	• Vision loss	<b>PPT1</b> (lysosomal lipid hydrolase)	Classical
	• Dementia	<b>TPP1</b> (lysosomal exopeptidase)	
	• Epilepsy	(Transmembrane protein)	
		(Soluble lysosomal protein)	
		(Transmembrane protein)	
		MFSD8 (transmembrane protein)	
		(Transmembrane protein)	
		<b>CtsD</b> (lysosomal peptidase)	
		ATP13A2 (ATPase)	
Young adults	• Nonspecific mental, motor, or behavioral abnormalities	<b>PPT1</b> (lysosomal lipid hydrolase)	Classical
		<b>TPP1</b> (lysosomal exopeptidase)	
		DNAJC5 (HSP40/DNAJ protein)	
		(autosomal dominant)	
		(Soluble lysosomal protein)	
		(Transmembrane protein)	
		<b>CtsD</b> (lysosomal peptidase)	
		GRN (progranulin)	
		CtsF (lysosomal proteinase)	

CLN, neuronal ceroid lipofuscinosis; Cts, cathepsin; GRN, granulin; HSP40, heat shock protein 40; KCTD7, potassium channel tetramerization domain containing 7; MFSD8, major facilitator superfamily domain containing 8; PPT1, palmitoyl-protein thioesterase 1; TPP1, tripeptidyl peptidase 1.

## Diagnostic Testing by Degree of CLN2 Disease Suspicion



**Fig. 1.** Diagnostic algorithm in support of early suspicion and diagnosis of neuronal ceroid lipofuscinosis type 2 (CLN2) disease. CLN2 disease is diagnosed by biochemical and molecular laboratory tests. Unexplained seizures, particularly when associated with a history of unexplained language delay and/or developmental milestone regression, may be caused by a neuronal ceroid lipofuscinosis (NCL) disorder; another common possible early symptom is ataxia. Electroencephalography (EEG) analysis under specific intermittent photic stimulation (IPS; 1–2 Hz) may assist with differential diagnoses. The recommended approach once CLN2 disease is specifically suspected (center) is demonstration of deficient tripeptidyl peptidase 1 (TPP1) enzyme activity in leukocytes together with the identification of 2 pathogenic mutations in the *TPP1/CLN2* gene. When an NCL disorder is suspected (right), an NCL gene panel and/or TPP1 enzyme screening along with palmitoyl-protein thioesterase 1 (PPT1) enzyme screening is recommended. When the main suspicion is broadly of a genetic basis of epilepsy (left), a gene panel to investigate genetic causes of childhood-onset epilepsy is recommended. CLN2 disease diagnosis is achieved upon demonstration of deficient TPP1 enzyme activity in leukocytes together with normal activity of  $\geq 1$  appropriate control enzyme (such as PPT1 and/or  $\beta$ -galactosidase) and identification of 2 pathogenic mutations in *trans* in the *TPP1/CLN2* gene. EM, electron microscopy; ERG, electroretinography; FA, fluorescein angiography; MRI, magnetic resonance imaging; OCT, optical coherence tomography; VEP, visually evoked potential.

Findings of brain imaging techniques (such as magnetic resonance imaging) include progressive cerebellar and cerebral atrophy, reductions in grey matter volume, and periventricular white matter hyperintensities [8,16–19]. It is important to recognize that although CLN2 is a grey matter disease, periventricular white matter hyperintensities may be seen and should not deviate the diagnosis path towards a leukodystrophy. These findings may provide clues for diagnosis.

EEG with intermittent photic stimulation (IPS) performed at a frequency of 1 to 2 Hz is a particularly informative test (Table 2). In many EEGs of those ultimately diagnosed with CLN2 disease a characteristic flash-per-flash response has been reported at low frequency of stimulation. A classical epileptiform photoparoxysmal response is usually evident at higher frequencies of stimulation [3,5,20–22]. Although IPS is often part of routine assessment performed in response to new-onset seizures, if performed only at frequencies of  $> 2$  Hz, the characteristic response (at 1–2 Hz) that is present in many individuals with CLN2 disease will be missed. Lack of a characteristic response cannot rule out CLN2 disease, however.

### 2.2. High suspicion of an NCL disorder

More commonly, a history of ataxia, worsening unprovoked seizures, and presence of early language delay may not specifically be recognized as CLN2 disease but may be generally suggestive of an NCL disorder upon presentation to a metabolic specialist/geneticist, an NCL

specialist, or a pediatric neurologist with experience diagnosing NCL disorders. Upon a general suspicion of an NCL disorder (Fig. 1, right), use of an NCL gene panel is endorsed to rapidly assess all known NCL genes, as most NCL gene products cannot readily be assessed in a biochemical assay. Equally highly recommended is assessment of enzyme activity of TPP1 and palmitoyl-protein thioesterase 1 (PPT1; EC 3.1.2.22) as a screening approach, because the associated diseases (CLN2 and CLN1 [OMIM 256730], respectively) are the most prevalent infantile and late-infantile NCL disorders [13], and reliable enzyme activity assays exist for both. Other investigations may include sequential sequencing of commonly mutated NCL genes and/or use of electron microscopy (EM) to assess the accumulation of intracellular storage materials, which is a general hallmark of the NCL disorders [13,23–28] (Table 2).

### 2.3. High suspicion of genetic basis for epilepsy/neurological symptoms

Most commonly, initial presentation of ataxia and/or unprovoked seizures and a history of a language delay to a pediatrician or even to a pediatric neurologist results in no specific suspicion of CLN2 disease or NCL. Seizures are a relatively common symptom that prompts medical consultation in young children ranging from 1% to 14% of children under 5 years globally depending on region [29–31]. As part of the investigation into the etiology of epileptic seizures, there is increasing evidence to support use of a gene panel to disclose a genetic basis of

**Table 2**  
Roles of recommended investigations in the diagnosis of CLN2 disease.

Nature	Test	Sample type(s)	Considerations	Role in diagnosis
Clinical test	EEG with IPS at 1 to 2 Hz	N/A	Individuals with CLN2 disease have characteristic EEG posterior spikes in response to each light flash (at 1–2 Hz) [5]  NCL disorders other than CLN2 disease may present with these characteristic responses  Note that IPS is often routinely performed at >2 Hz, which will not elicit these characteristic responses	Not a diagnostic test
Electron microscopy	Detection of intracellular storage bodies	Typically skin, rectal, or blood samples	Accurate assessment and interpretation require experience and specialized skills that are not widely available  Findings may not be unique to a specific NCL	Not sufficient for diagnosis of a specific NCL disorder
Enzyme assay	TPP1 enzyme activity assay	DBS or saliva	Always assess activity of $\geq 1$ enzyme in addition to TPP1 (PPT1 and/or $\beta$ -galactosidase recommended)	Deficient TPP1 activity in DBS or saliva is diagnostic if confirmed by molecular analysis
Enzyme assay	TPP1 enzyme activity assay	Leukocytes or fibroblasts	Always assess activity of $\geq 1$ enzyme in addition to TPP1 (PPT1 and/or $\beta$ -galactosidase recommended)	Deficient TPP1 activity in leukocytes or fibroblasts is diagnostic if consistent with clinical presentation  To confirm a diagnosis, molecular analysis is recommended
Molecular analysis	Gene panels containing the <i>TPP1/CLN2</i> gene	Typically blood, buccal swabs, or saliva; other samples possible	Does not require specific suspicion of CLN2 disease, potentially speeding path to diagnosis  Sanger sequencing is recommended to confirm identified mutations  Access and reimbursement can vary regionally  Analysis of parental DNA samples may clarify whether detected alterations are in <i>trans</i> or in <i>cis</i>  Not all detected alterations may be readily interpretable, and in rare cases, both causative mutations may not be identified	Findings not diagnostic unless test is validated for diagnosis  If validated: a finding of 2 pathogenic mutations consistent with clinical presentation in <i>trans</i> is diagnostic  To confirm a diagnosis, enzyme analysis is necessary if only 1 pathogenic variant is found, no variant is found, or if 1 or more VUS is identified
Molecular analysis	<i>TPP1/CLN2</i> gene sequencing	Typically blood, buccal swabs, or saliva; other samples possible	Sequencing of all exons and associated intron-exon junctions recommended  Analysis of parental DNA samples may clarify whether detected alterations are in <i>trans</i> or in <i>cis</i>  Not all detected alterations may be readily interpretable, and in rare cases, both causative mutations may not be identified	A finding of 2 pathogenic mutations consistent with clinical presentation in <i>trans</i> is diagnostic  To confirm a diagnosis, enzyme analysis is necessary if only 1 pathogenic variant is found, no variant is found, or if 1 or more VUS is identified

CLN2, neuronal ceroid lipofuscinosis 2; DBS, dried blood spot; EEG, electroencephalogram; IPS, intermittent photic stimulation; N/A, not applicable; PPT1, palmitoyl-protein thioesterase 1; TPP1, tripeptidyl peptidase 1; VUS, variant of unknown/uncertain significance.

epilepsy for childhood, late-infantile, or particularly infantile epileptic seizures. Therefore, at this stage, the clinician (Fig. 1, left) may order a gene panel directed to epilepsy and seizure disorders [32–35]. Whole exome and whole genome sequencing may also be considered to uncover a previously uncharacterized genetic basis of epilepsy.

#### 2.4. Differential diagnosis of CLN2 disease

Different epilepsy syndromes may be considered in young children with new-onset seizures, including: Ohtahara, West, Dravet, and Lennox-Gastaut, myoclonic-astatic epilepsy/MAE (Doose syndrome), and Landau-Kleffner syndromes. GLUT1 deficiency, benign myoclonic epilepsies, progressive myoclonic epilepsies (Lafora disease, Unverricht-Lundborg disease, myoclonic epilepsy with ragged-red fibers), along with other channelopathies and metabolic syndromes that are associated with myoclonic epilepsy (including sialidoses and galactosialidosis) also may be considered.

Epilepsy and progressive neurodegeneration in children should raise suspicion of not only NCL, but also gangliosidoses, mucopolysaccharidoses,

mucopolysaccharidoses, Niemann-Pick type C disease, peroxisomal disorders, mitochondrial disorders, Gaucher disease type III, and leukodystrophies.

While many of these disorders and syndromes are in fact distinguishable from the classic presentation of CLN2 disease, natural variation in clinical presentation of these disorders and syndromes often conceals an early and direct path to diagnosis of CLN2 disease. Distinguishing between these disorders and syndromes is difficult as many lack biochemical diagnostic tests, such as enzyme activity tests and/or biomarker tests, slowing the time to diagnosis. Fortunately, there is a biochemical test for diagnosis of CLN2 disease.

### 3. Laboratory diagnostic tests for CLN2 disease

#### 3.1. TPP1 enzyme activity analysis

CLN2 disease is diagnosable by a biochemical test of TPP1. TPP1 enzyme is a pepstatin-insensitive lysosomal serine exopeptidase with optimal *in vitro* activity at acidic pH [1,36–38]. Although a tandem mass

spectrometry (MS) compatible substrate was recently developed [39], most diagnostic laboratories assess TPP1 enzyme activity using the fluorogenic substrate, Ala-Ala-Phe-7-amido-4-methylcoumarin [37,38,40]. Several variations on the fluorogenic methodology have been published [41–43], each of which can differentiate affected individuals from healthy controls; however, the absolute activities differ. Therefore, it is essential that laboratories carefully establish unaffected reference ranges for their implementation of the TPP1 enzyme assay (Fig. 2).

Leukocytes isolated from whole blood are the recommended sample type for analysis of TPP1 enzyme activity, enabling accurate and rapid diagnostic analysis. Multiple laboratories report TPP1 enzyme activity levels measured in affected individuals to be distinct from carriers and unaffected individuals (although the absolute activities differ; Fig. 2). However, whole blood samples are particularly sensitive to exposure to temperature extremes or to delays in shipping and receipt, potentially reducing assay reliability.

In addition to leukocytes, several other sample types may be used for analysis of TPP1 enzyme activity, such as fibroblasts, dried blood spots (DBS), and saliva. The TPP1 enzyme assay should be separately validated for each sample type in which it is performed. Although analysis of TPP1 enzyme activity in fibroblasts requires culture of a skin punch biopsy, leading to delays in diagnostic testing, skin punch biopsies are more robust in suboptimal shipment conditions than whole-blood samples. DBS samples are of great use in regions where the timely and appropriate shipment of blood and/or tissue samples is problematic and is the likely sample of choice for any screening approaches to detect CLN2 disease [39], although appropriate DBS sample collection, drying, and timely shipment remain important for DBS samples. Evaluation of TPP1 enzyme activity in saliva samples [44] is a component of an integrated strategy for diagnosis of NCL disorders in Latin America [10,26].

Appropriate control enzymes are essential to accurately interpret TPP1 enzyme activity findings. PPT1 is a suitable control enzyme because it has broadly similar stability as the TPP1 enzyme in DBS [45], and the experience of most investigators is that the stabilities are comparable in leukocytes, fibroblasts, and saliva. Additionally, evaluation of PPT1 enzyme activity serves a dual function to both assess sample quality and exclude

CLN1 disease, another NCL disorder that can initially present with seizures at similar ages as those with CLN2 disease. An additional appropriate control enzyme is  $\beta$ -galactosidase (EC 3.2.1.23), a lysosomal enzyme not associated with an NCL disorder. Currently, the only other lysosomal enzyme defect associated with NCL disease, and for which a specific clinical test is available, is cathepsin D (CLN10 disease). Whereas, the remainder of the NCL disorders are not due to an enzyme deficiency.

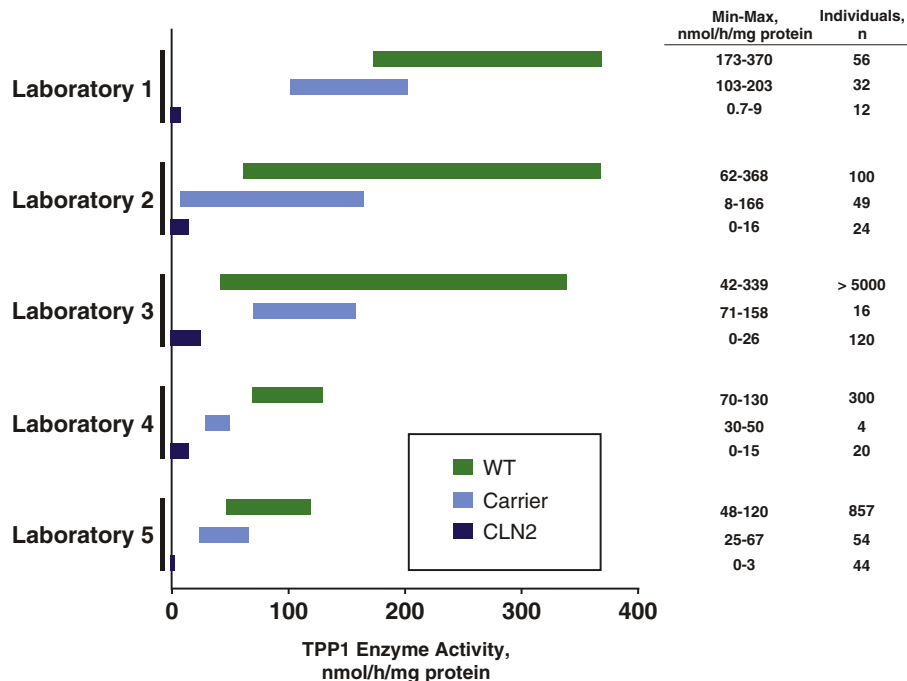
### 3.2. Electron microscopy (EM) analysis

Accumulation of intracellular storage materials in neuronal and non-neuronal cells is a morphological hallmark of the NCL disorders. Historically, detection of intracellular storage materials by EM has been an important technique for the classification and diagnosis of NCL disorders [46]. The recent increase in availability of molecular testing has limited the use of EM studies in the diagnosis and differentiation of NCL disorders [23]. In addition, few laboratories now have the required expertise for the accurate interpretation of electron micrographs.

Clinical EM investigation of those suspected of having an NCL disorder typically requires collection of skin biopsies or blood samples; rectal, skeletal muscle, and conjunctival biopsies can also be used [23]. EM examination shows accumulation of storage materials, typically with curvilinear profiles in CLN2 disease. Some CLN2 disease biopsies show a mixed pattern of both curvilinear and fingerprint profiles, a pattern that may be associated with atypical CLN2 phenotypes [10,23,28].

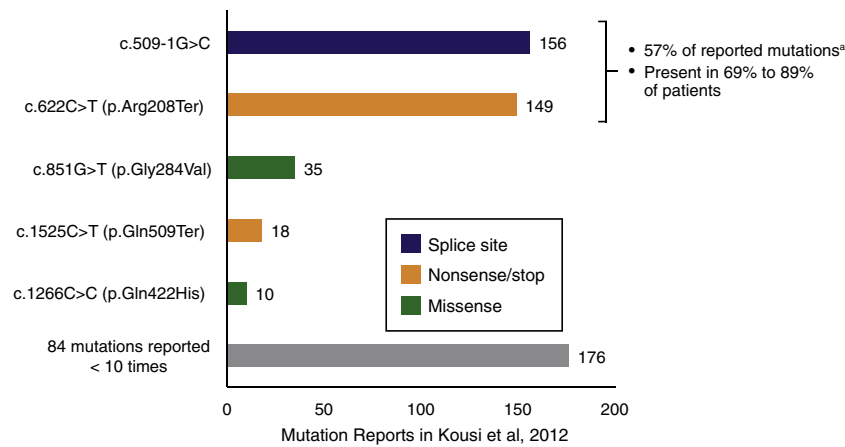
### 3.3. Molecular analysis of TPP1/CLN2

A diagnosis of CLN2 disease can be confirmed by identification of two pathogenic variants/mutations (associated with a TPP1 enzyme deficiency) in *trans* in the *TPP1/CLN2* gene. The *TPP1/CLN2* gene is located on chromosome 11p15 [47], contains 13 exons, and is 6.7 kb in length. As of March 2016, 140 changes in the *TPP1/CLN2* gene have been reported, of which 116 are reported to be pathogenic [13,28,48]. Globally, the two most commonly reported mutations associated with CLN2 disease are c.509-1G>C, a splicing mutation, and c.622C>T (p.Arg208Ter),



**Fig. 2.** Enzyme activity ranges of tripeptidyl peptidase 1 (TPP1) in leukocytes observed in 5 independent laboratories. Activity ranges for healthy individuals (wild type [WT]), carriers, and affected individuals (with neuronal ceroid lipofuscinosis type 2 [CLN2] disease) are reported. Although the absolute enzyme activities differ among the laboratories (likely due to methodological differences, such as the protease inhibitors used and the assay pH), each individual laboratory clearly differentiates the TPP1 enzyme activity of individuals with CLN2 disease from that of unaffected controls.





\* as reported in Kousi et al, 2012

**Fig. 3.** Mutations associated with classic neuronal ceroid lipofuscinosis type 2 (CLN2) disease. Five mutations in the tripeptidyl peptidase 1 (*TPP1*)/*CLN2* gene have been reported > 10 times [28]. The mutations c.509-1G>C and c.622C>T (p.Arg208Ter) together represent 57% of all reported mutations (reported by Kousi et al. [28]), and ≥ 1 of these 2 alleles is present in 69% to 89% of those diagnosed with CLN2 disease [28,49,50].

which results in the introduction of a premature termination codon [13, 28,49,50] (Fig. 3). However, in one laboratory's experience, these two alleles have not been identified during diagnosis of individuals of Southeast Asian or Middle Eastern origin (M. Fietz, written communication, April 2016), indicating that c.509-1G>C and c.622C>T (p.Arg208Ter) are not common in all populations. Further, different mutation profiles have been reported in some locations, such as Argentina [10,25,26] and Canada/Newfoundland, where a founder effect mutation has been well characterized [28,51,52]. Consequently, global allele frequencies are not necessarily relevant for some local diagnostic purposes.

Given the broad range of mutations that have been associated with CLN2 disease [13,28], sequencing of the *TPP1/CLN2* gene should ideally evaluate the entire coding region and associated intron–exon splice junctions. If DNA sequencing fails to identify mutations, other approaches such as mRNA analysis, array comparative genome hybridization (aCGH), deletion/duplication analyses, and whole genome/exome sequencing may be of use. If two pathogenic mutations in the *TPP1/CLN2* gene are identified, it is important to confirm that they are on separate parental alleles (*i.e.*, *in trans*); analysis of parental DNA samples can clarify the phase of the detected mutations. Analysis of parental DNA can also detect possible allele dropout and subsequent false assessments of homozygosity. *De novo* mutations that arise in germ cells are also possible, although this has not been reported to date. It has been the experience of the authors that both *TPP1/CLN2* mutations are generally identified by DNA sequencing and, to date, few large deletions have been identified in individuals with CLN2 disease. In cases where one *TPP1/CLN2* gene pathogenic variant/mutation is uncovered in molecular testing, enzyme activity testing is warranted as detection of only one pathogenic variant cannot rule out CLN2 disease.

As with other recessive genetic disorders, molecular analysis may identify variants of unknown significance (VUS). At present, the number of reported VUS and likely non-pathogenic sequence changes in the *TPP1/CLN2* gene is relatively low: of 140 reported sequence alterations, 24 alterations (17%) are not believed to be pathogenic [13,48]. However, it is likely that not all detected VUS have been reported. The increasing use of molecular testing in suspected cases of CLN2 disease may increase the number of VUS. Again, if two pathogenic mutations in *trans* are not identified, diagnostic testing of TPP1 enzyme activity is required.

#### 3.4. Gene panels and whole exome/genome sequencing

Relevant gene panels—such as symptom-based (epilepsy) gene panels, NCL gene panels, lysosomal disorder or inherited metabolic

disorder gene panels—may speed the diagnostic process when CLN2 disease is not specifically suspected. Key strengths of gene panels include relatively rapid turnaround time, the assessment of many genetic conditions in one analysis, and the investigation of genetic conditions that are not diagnosed through enzyme activity assays as mentioned. An informal survey of US laboratories offering gene panels suggests that the *TPP1/CLN2* gene is present on many gene panels, including those targeting NCL disorders, epilepsy, neurology, lysosomal storage disorders, inherited metabolic disorders, and eye disorders. The authors recommend the use of gene panels given the support they can provide clinicians in moving rapidly from clinical presentation to a laboratory diagnosis.

Whole exome/genome sequencing (WEGS) approaches share many of the strengths of gene panels and have the advantage of examining far more of the genome, albeit with an accompanying increase in data complexity and analysis requirements. WEGS approaches may be of particular use for identification of novel diseases or if the clinical manifestation of a given mutation is outside of a gene's known disease spectrum, as was the case for the *TPP1/CLN2* mutations found to cause SCAR7 disease [12]. However, not all regions will have access or support for WEGS approaches, and the cost and turnaround time relative to a gene panel is typically higher and longer.

#### 4. Conclusions

In early stages, CLN2 disease is challenging to recognize, and diagnosis is often delayed until after the disease has progressed significantly. In the majority of cases, key initial symptoms are new-onset epileptic seizures in combination with a history of early language delay and/or ataxia, although alternative presentations are possible [5]. Differential diagnosis of a genetic basis of epilepsy by use of symptom- or disease-based gene panels offer great promise for supporting an early and timely diagnosis of not only CLN2 disease but many other causes of late-infantile epilepsy as well. In the absence of newborn screening for CLN2 disease, gene panels are the broadest symptom-based, multiple-disease screening approach with a reasonable diagnostic yield. Laboratory tests to diagnose CLN2 disease are well established (Table 2). TPP1 enzyme activity can be assessed in several sample types: leukocytes, DBS, fibroblasts, and saliva. The gold standard for laboratory diagnosis is demonstration of deficient TPP1 enzyme activity (in conjunction with normal activity of a control enzyme such as PPT1 and/or β-galactosidase) followed by molecular analysis that detects one pathogenic mutation on each parental allele of *TPP1/CLN2*. Given the availability of reliable

enzyme activity assays for both TPP1 and PPT1, together with the higher prevalence of the NCL disorders for which TPP1 or PPT1 activity is lost (CLN2 disease and CLN1 disease, respectively [13]), we recommend that the activity of each enzyme be assayed early in any individual suspected of having an NCL disorder. Future prospects for early diagnosis include ongoing development of MS-compatible enzyme substrates [39], which could support large-scale screening approaches such as newborn screening. Disease-specific management, genetic counseling, and new therapies in development for CLN2 disease make early and accurate diagnosis of this severe neurodegenerative disease essential.

### Conflicts of interest

M. Fietz has received honoraria and travel support from BioMarin and Genzyme. M. AlSayed, D. Burke, L. Dvořáková, H. Jahnová, and W. Xin have received honoraria and travel support from BioMarin. J. Cohen-Pfeffer, E. Izzo, and N. Miller are employees and shareholders of BioMarin. J. D. Cooper has received honoraria, travel support, and consulting fees from BioMarin; has received consultant fees from Stem Cell Inc.; and receives research funding from BioMarin. R. Giugliani has received travel support, speaker honoraria, and investigator support from BioMarin. Z. Lukacs has received honoraria, travel support, and research funding from BioMarin. S. E. Mole has received honoraria from BioMarin and serves without compensation as a scientific advisor for the Batten Disease Family Association UK. I. Noher de Halac has received honoraria and travel support from BioMarin, has received research funding from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and the Secretaría de Ciencia y Técnica (SeCYT) of the Universidad Nacional de Córdoba, and receives research funding from the Ministerio de Ciencia, Tecnología e Innovación Productiva Fondo para la Investigación Científica y Tecnológica. D. A. Pearce and H. Poupetova declare that they have no conflict of interest. A. Schulz is a consultant for and has received a research grant from BioMarin. N. Specchio is a consultant for BioMarin.

### Author contributions

All authors were involved in the conception, drafting, and review of this manuscript.

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