THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Next Generation Sequencing Panel for Neuronal Ceroid-Lipofuscinoses

Clinical Features:

The neuronal ceroid-lipofuscinoses (NCLs) are a clinically and genetically heterogenous group of inherited neurodegenerative lysosomal storage disorders associated with intellectual and motor deterioration, seizures, and early death [1]. Loss of vision is also a feature of most forms of NCL [1]. Subtypes of the NCLs are classified by age of onset, which can be congenital, infantile, late infantile, juvenile (also known as Batten disease), or adult [1]. Infantile, late infantile and adult NCLs often present with seizures as their first symptom [1]. The first symptom of juvenile NCL is more likely to be rapid loss of vision [1]. Intracellular accumulation of autofluorescent lipopigment storage material can be observed by doing electron microscopy (EM) studies on tissue or lymphocytes; subtypes of NCL may be differentiated by different ultra-structural patterns seen on EM.

*Our Neuronal Ceroid-Lipofuscinoses Panel includes sequence analysis of all 11 genes listed below, and deletion/duplication analysis of the 8 genes listed in **bold**.*

Neuronal Ceroid-Lipofuscinoses Panel					
CLN3	**CLN6**	**CTSD**	DNAJC5	**MFSD8**	**TPP1**
CLN5	**CLN8**	CTSF	GRN	**PPT1**	

Gene	Disorder	Clinical Features
CLN3 [OMIM#607042]	CLN3 [OMIM#204200]	Mutations in *CLN3* are the main cause of classic juvenile NCL [1]. The first symptom is typically insidious onset of retinitis pigmentosa at age 4-6 years, followed by progressive cognitive decline and seizures [2]. Other symptoms may include myoclonus, parkinsonism, severe dysarthria and behavioral issues with angry outbursts and physical violence [2]. *CLN3* mutations may also be associated with adult NCL in some cases [1]. The CLN3 protein localizes to the lysosomes, and has been hypothesized to play a role in regulating neuronal cell death [3].
CLN5 [OMIM#608102]	CLN5 [OMIM#256731]	Mutations in *CLN5* are associated with the Finnish variant of late infantile NCL. The disorder was first identified in 18 Finnish families of common ancestry, with features including intellectual disability, ataxia and myoclonic epilepsy [4]. Age of onset is typically around age 4-7 years [4]. Mutations in *CLN5* have since been described outside of the Finnish population. *CLN5* mutations may also be associated with adult NCL in some cases [1]. *CLN5* is a lysosomal protein, and mutations in this gene are associated with defective lysosomal trafficking [5].
CLN6 [OMIM#607042]	CLN6 [OMIM#601780]	*CLN6* mutations are associated with the "Early Juvenile" variant of late infantile NCL. Age of onset is typically around between 1-8 years, and presenting symptoms typically include vision loss and seizures [1]. For children with age of onset after 4 years, epilepsy, ataxia and myoclonus may be the presenting features [1]. Studies of *CLN6* mouse models revealed changes in the expression levels of proteins involved in synaptic function and stability and cell-cycle regulation [6].
CLN8 [OMIM#601780]	CLN8 [OMIM#600143]	Mutations in the *CLN8* gene can be associated with either the "Northern Epilepsy" variant of NCL (NE), or late infantile NCL [1]. The NE phenotype is characterized by tonic-clonic or complex-partial seizures, intellectual decline and motor dysfunction [1]. There is a specific mutation in *CLN8* which is associated with the NE phenotype, which has a founder effect in the Finnish population [1]. CLN8 is a transmembrane protein in the endoplasmic reticulum, and functional studies have found that neuronal cellular proliferation is increased in cell lines with *CLN8* mutations [7].
CTSD [OMIM#116840]	CLN10 [OMIM#610127]	*CTSD* mutations have been identified in patients with congenital NCL, which is the most severe NCL subtype [8]. Findings include microcephaly, seizures, extensive neural loss, gliosis and early death [8]. *CTSD* mutations may also be observed in other subtypes of NCL, including late infantile, juvenile and adult [1, 8]. *CTSD* encodes for a lysosomal proteinase involved in proteolytic degradation, cell invasion and apoptosis. It has been hypothesized that patients who retain some residual enzyme activity have a less severe phenotype and a later age of onset than those with complete loss of activity [8].
CTSF [OMIM#603539]	CLN13 [OMIM#615362]	Mutations in *CTSF* are associated with adult onset NCL, known as CLN13 or Kufs disease type B. Features include seizures, ataxia, dysarthria, cerebellar and cortical atrophy, progressive cognitive decline, and progressive dementia [9]. The *CTSF* gene encodes cathepsin F, a cysteine protease involved in lysosomal proteolytic activity. Tang, *et al.* (2006) found that deficiency of cathepsin F in mice leads to neuronal lipofuscinosis and late-onset neurological disease [10].
DNAJC5	CLN4	Heterozygous mutations in *DNAJC5* have been described in families with an

[OMIM#611203]	[OMIM#162350]	autosomal dominant form of NCL with symptoms arising in adulthood. Features include seizures, myoclonus, cognitive decline and dementia. Burneo, *et al.* (2003) reported a family with CLN4 in which multiple individuals exhibited parkinsonism [11]. Missense and frameshift mutations have been reported [12].
GRN [OMIM#138945]	CLN11 [OMIM#614706]	Smith, *et al.* (2012) identified a homozygous frameshift mutation in *GRN* in two siblings with neuronal ceroid lipofuscinosis [13]. These individuals exhibited onset of symptoms in their early 20s, with retinal dystrophy, seizures, myoclonus, and cerebellar atrophy [14]. Both patients had normal cognitive functioning at the time of evaluation, at which time they were in their mid to late 20s. Electron microscopy on peripheral blood leukocytes in these patients revealed fingerprint profiles in 1/100 lymphocytes [14]. Heterozygous mutations in *GRN* are associated with frontotemporal lobar degeneration with TDP43 inclusions (FTLD-TDP) [OMIM# 607485].
MFSD8 [OMIM#611124]	CLN7 [OMIM#610951]	Mutations in *MFSD8* are associated with a variant of late infantile NCL [15]. Mutations were first identified in Turkish patients who presented with seizures and/or motor impairment at an average age of 5 years [15]. The patients later developed developmental regression, myoclonus, speech impairment, vision loss and personality disorders [15]. The *MFSD8* protein localizes to the lysosome and functions as a transporter protein, however further details regarding the function of the MFSD8 protein are yet to be elucidated [15].
PPT1 [OMIM#600722]	CLN1 [OMIM#256730]	*PPT1* mutations have been described in association with several different subtypes of NCL, including infantile, late infantile, juvenile and adult [1]. *PPT1* encodes a lysosomal enzyme with "housekeeping" role, it has also been hypothesized to play a role in synaptic functioning [1, 16].
TPP1 [OMIM#607998]	CLN2 [OMIM#204500]	Mutations in *TPP1* are typically associated with classic late infantile NCL, and age of onset is typically around 2-4 years. Presenting symptoms are usually seizures, cognitive decline and vision loss [1, 17]. TPP1 is also a rare cause of JNCL [1]. TPP1 encodes for a serine-carboxyl peptidase that localizes to the lysosome [1].

Inheritance:

The NCLs are typically inherited in an autosomal recessive pattern. Parents of an affected child are most likely obligate carriers. Recurrence risk for carrier parents is 25%. Adult NCL, which can be associated with mutations in *CLN3, CLN5, CLN6, CTSD* and *PPT1*, can be inherited in either an autosomal recessive or autosomal dominant pattern. Most individuals with autosomal dominant adult NCL have an affected parent. The proportion of *de novo* cases is unknown. Recurrence risk for affected individuals with autosomal dominant adult NCL is 50%.

Test methods:

Comprehensive sequence coverage of the coding regions and splice junctions of all genes in this panel is performed. Targets of interests are enriched and prepared for sequencing using the Agilent SureSelect system. Sequencing is performed using Illumina technology and reads are aligned to the reference sequence. Variants are identified and evaluated using a custom collection of bioinformatic tools and comprehensively interpreted by our team of directors and genetic counselors. All pathogenic and likely pathogenic variants are confirmed by Sanger sequencing. The technical sensitivity of this test is estimated to be >99% for single nucleotide changes and insertions and deletions of less than 20 bp. Deletion/duplication analysis of the panel genes is performed by oligonucleotide array-CGH. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by array-CGH. Array-CGH will not detect low-level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of this assay may be reduced when DNA is extracted by an outside laboratory.

Neuronal Ceroid-Lipofuscinoses Panel (sequence analysis of 11 genes and deletion/duplication analysis of 8 genes)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$2000
CPT codes:	81406, 81407
Turn-around time:	8 weeks

Note: We cannot bill insurance for the Neuronal Ceroid-Lipofuscinoses panel

Note: The sensitivity of our assay may be reduced when DNA is extracted by an outside laboratory.

Results:

Results, along with an interpretive report, are faxed to the referring physician as soon as they are completed. One report will be issued for the entire Neuronal Ceroid-Lipofuscinoses Panel. All abnormal results are reported by telephone.

For more information about our testing options, please visit our website at dnatesting.uchicago.edu or contact us at 773-834-0555.

References:

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