THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Next Generation Sequencing Panel for NBIA

Clinical Features:
NBIA (Neurodegeneration with Brain Iron Accumulation) is a group of genetic disorders characterized by iron accumulation in the basal ganglia. Brain iron accumulation can result in progressive dystonia, spasticity, parkinsonism, neuropsychiatric abnormalities, and optic atrophy or retinal degeneration (1). A minority of subtypes are associated with cognitive decline. Age of onset varies from infancy to late adulthood (1).

Our NBIA Panel includes analysis of all 10 genes listed below.

	NBIA Sequencing Panel								
ATP13A2	C19orf12	COASY	CP	DCAF17	FA2H	FTL	PANK2	PLA2G6	WDR45

Gene / Condition	Clinical and Molecular Findings
ATP13A2 Kufor-Rakeb syndrome	Mutations in *ATP13A2* are associated with Kufor-Rakeb syndrome, which is characterized by juvenile-onset parkinsonism, dementia, supranuclear gaze palsy, pyramidal signs, visual hallucinations and oculogyric dystonic spasms (1, 2). Some affected individuals have been described with evidence of brain iron accumulation in the caudate and putamen, however this not observed in all patients (2). *ATP13A2* encodes for an ATPase that transports inorganic cations and other substrates across cell membranes, and is most strongly expressed in the brain (3).
C19orf12 MPAN (Mitochondrial Membrane Protein-Associated Neurodegeneration)	MPAN is caused by mutations in the *C19orf12* gene, and is associated with motor neuronopathy, spasticity, dystonia, optic atrophy and neuropsychiatric abnormalities (1, 4). Most patients also have cognitive decline. Onset of symptoms is typically in childhood or early adulthood, and the disorder is slowly progressive (1). Iron accumulation occurs in all patients and exhibits a distinctive pattern involving the globus pallidus and substantia nigra (1, 4). *C19orf12* encodes a protein that localizes to the mitochondria and is expressed most prominently in the brain, blood cells and adipocytes (5).
COASY Neurodegeneration with brain iron accumulation-6	Biallelic mutations in *COASY* have been identified in two unrelated Italian patients with NBIA. Both patients were reported to have normal psychomotor development until 2 years of age, when they developed difficulty walking. This progressed to spastic paraplegia resulting in wheelchair use, cognitive impairment and behavioral abnormalities. The brain imaging in both patients was consistent with the 'eye of the tiger' sign (6). The COASY enzyme catalyzes the last two steps in Coenzyme A (CoA) synthesis. Loss of function mutations in this gene lead to decreased CoA production. COASY is the second enzyme related to CoA biosynthesis to be implicated in NBIA, after *PANK2*.
CP Aceruloplasminemia	Mutations in *CP* cause Aceruloplasminemia, which is associated with retinal degeneration, diabetes and neurological disease (7 Apr 18] #664). Neurological manifestations can include facial and neck dystonia, blepharospasm, grimacing, tremors, chorea, gait ataxia and dysarthria (7 Apr 18] #664). Age of onset ranges from early to late adulthood. Iron accumulation typically occurs in the striatum, thalamus and dentate nucleus of the brain, as well as visceral organs (7 Apr 18] #664). Affected individuals also have low serum copper and iron, and high serum ferritin. *CP* encodes for the precursor to ceruloplasmin, which transports copper and also plays an important role in iron mobilization (7 Apr 18] #664).
DCAF17 Woodhouse-Sakati syndrome	Woodhouse-Sakati syndrome is associated with mutations in *DCAF17*, and is characterized by hypogonadism, partial alopecia, diabetes, intellectual disability, deafness and a progressive extrapyramidal disorder (1, 8). Brain iron accumulation occurs in the globus pallidus, substantia nigra and other regions of the basal ganglia, and white matter disease is also frequently observed (1). *DCAF17* encodes a nucleolar protein of unknown function (8).
FA2H FAHN (Fatty Acid Hydroxylase-	FAHN is caused by mutation in the *FA2H* gene, and is associated with spasticity, ataxia and dystonia. Age of onset is typically in childhood, and affected individuals typically develop optic atrophy and oculomotor abnormalities during the early stages of disease, and progressive intellectual impairment and seizures in the later stages. Iron accumulation occurs in the globus

Associated Neurodegeneration)	pallidus and substantia nigra. Progressive white matter changes and cerebellar and brain stem atrophy are also observed. *FA2H* encodes for fatty acid 2-hydroxylase, which alpha-hydroxylates fatty acids, which are then incorporated into several diverse lipid species.
FTL Neuroferritinopathy	Mutations in *FTL* cause neuroferritinopathy, which is characterized by extrapyramidal features similar to Huntington disease or parkinsonism. The average age of onset is 39 years (9). Additional symptoms can include tremor, cerebellar ataxia, and cognitive decline. Affected individuals typically have low serum ferritin levels, and brain MRI findings consistent with excess iron storage and cystic changes involving the globus pallidus and the putamen (10). *FTL* encodes for a subunit of ferritin, and mutations in *FTL* cause abnormally shaped ferritin molecules and aggregation of ferritin and associated iron molecules in the brain (11).
PANK2 PKAN (Pantothenate Kinase-Associated Neurodegeneration)	PKAN (previously known as Hallervorden-Spatz syndrome) is caused by mutations in *PANK2*. There is wide phenotypic variability, including classic PKAN and atypical PKAN. Classic PKAN is associated with early onset (3-4 years of age), rapid progression, impaired gait, restricted visual fields, dystonia, dysarthria, spasticity, retinopathy, and possible cognitive impairment (12). Atypical PKAN has a mean age of onset of 13-14, slow progression, speech difficulties, neurobehavioral changes, Parkinson-like syndrome, spasticity, hyperreflexia and possible cognitive impairment (12). Most individuals with *PANK2* mutations show brain iron accumulation, which is specific to the globus pallidus and substantia nigra and appears as the "eye of the tiger" sign (12). *PANK2* is a key regulatory enzyme in several metabolic pathways of Coenzyme A biosynthesis (13).
PLA2G6 PLAN (PLA2G6-Associated Neurodegeneration)	PLAN is caused by mutations in *PLA2G6* and is associated with a continuum of three phenotypes: classic infantile neuroaxonal dystrophy (INAD), atypical neuroaxonal dystrophy (atypical NAD), and *PLA2G6*-associated dystonia-parkinsonism (14). Patients with INAD and atypical NAD both have hypointensity of the globus pallidus on T2-weighted MRI, indicating iron accumulation (14). INAD patients also have cerebellar gliosis, white matter abnormalities and a thin, vertically oriented corpus callosum (14). Patients with *PLA2G6*-associated dystonia-parkinsonism may show iron accumulation in the globus pallidus, substantia nigra and/or striatum, however this is often not evident until late in the disease course (14). *PLA2G6* encodes for an enzyme that plays an important role in cell membrane homeostasis and phospholipid metabolism, and *PLA2G6* mutations gene may result in phospholipase A2 dysfunction critical in brain iron regulation and normal axonal pathology (14).
WDR45 BPAN (Beta-Propeller Protein-Associated Neurodegeneration)	Mutations in *WDR45* cause BPAN, which is associated with global developmental delay in childhood and sudden onset of progressive dystonia-parkinsonism and dementia in adolescence or adulthood (15). Brain iron accumulation occurs in the globus pallidus and substantia nigra (15). *WDR45* encodes for a protein which has an important role in autophagy, and mutations in the *WDR45* gene leads to an accumulation of aberrant early autophagic structures (15).

Inheritance:

ATP13A2, C19orf12, CP, DCAF17, FA2H, PANK2 and *PLA2G6* mutations are inherited in an autosomal recessive pattern. Parents of an affected child are most likely obligate carriers. Recurrence risk for carrier parents is 25%. *FTL* mutations are inherited in an autosomal dominant pattern, and recurrence risk for children of affected individuals is 50%. Mutations in *WDR45* are inherited in an X-linked manner, and are typically *de novo*. The majority individuals affected by a *WDR45* mutation have been females, suggesting that these mutations may be lethal in most males.

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 novel and/or potentially 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. Our CNV detection algorithm was developed and its performance determined for the sole purpose of identifying deletions and duplications within the coding region of the gene(s) tested. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by this methodology. Regions of high homology and repetitive regions may not be analyzed. This methodology will not detect low level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of our deletion/duplication assay may be reduced when DNA extracted by an outside laboratory is provided.

NBIA Panel (10 genes)

Sample specifications: 3 to10 cc of blood in a purple top (EDTA) tube

Cost:	$2,500
CPT codes:	81406
	81407
Turn-around time:	8 weeks

Note: We cannot bill insurance for the above test.

Results:

Results, along with an interpretive report, will be faxed to the referring physician. Additional reports will be provided as requested. All abnormal results will be 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:

1. Gregory A, Hayflick S. Neurodegeneration with Brain Iron Accumulation Disorders Overview.: GeneReviews [Internet]. Seattle, WA: University of Washington, Seattle., 2013, Feb 28.
2. Kruer MC, Paudel R, Wagoner W et al. Analysis of ATP13A2 in large neurodegeneration with brain iron accumulation (NBIA) and dystonia-parkinsonism cohorts. Neurosci Lett 2012: 523: 35-38.
3. Ramirez A, Heimbach A, Gründemann J et al. Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. Nat Genet 2006: 38: 1184-1191.
4. Hogarth P, Gregory A, Kruer MC et al. New NBIA subtype: genetic, clinical, pathologic, and radiographic features of MPAN. Neurology 2013: 80: 268-275.
5. Hartig MB, Iuso A, Haack T et al. Absence of an orphan mitochondrial protein, c19orf12, causes a distinct clinical subtype of neurodegeneration with brain iron accumulation. Am J Hum Genet 2011: 89: 543-550.
6. Dusi S, Valletta L, Haack TB et al. Exome sequence reveals mutations in CoA synthase as a cause of neurodegeneration with brain iron accumulation. Am J Hum Genet 2014: 94: 11-22.
7. Miyajima H. Aceruloplasminemia.: GeneReviews [Internet]. Seattle, WA: University of Washington, Seattle., 2003, Aug 12 [Updated 2013, Apr 18].
8. Alazami AM, Al-Saif A, Al-Semari A et al. Mutations in C2orf37, encoding a nucleolar protein, cause hypogonadism, alopecia, diabetes mellitus, mental retardation, and extrapyramidal syndrome. Am J Hum Genet 2008: 83: 684-691.
9. Chinnery PF, Crompton DE, Birchall D et al. Clinical features and natural history of neuroferritinopathy caused by the FTL1 460InsA mutation. Brain 2007: 130: 110-119.
10. Curtis AR, Fey C, Morris CM et al. Mutation in the gene encoding ferritin light polypeptide causes dominant adult-onset basal ganglia disease. Nat Genet 2001: 28: 350-354.
11. Levi S, Cozzi A, Arosio P. Neuroferritinopathy: a neurodegenerative disorder associated with L-ferritin mutation. Best Pract Res Clin Haematol 2005: 18: 265-276.
12. Hayflick SJ, Westaway SK, Levinson B et al. Genetic, clinical, and radiographic delineation of Hallervorden-Spatz syndrome. N Engl J Med 2003: 348: 33-40.
13. Zhou B, Westaway SK, Levinson B et al. A novel pantothenate kinase gene (PANK2) is defective in Hallervorden-Spatz syndrome. Nat Genet 2001: 28: 345-349.
14. Gregory A. PLA2G6-Associated Neurodegeneration.: GeneReviews [Internet]. Seattle, WA: University of Washington, Seattle., 2008, Jun 18 [Updated 2012 Apr 19].
15. Saitsu H, Nishimura T, Muramatsu K et al. De novo mutations in the autophagy gene WDR45 cause static encephalopathy of childhood with neurodegeneration in adulthood. Nat Genet 2013: 45: 445-449, 449e441.

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