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 Sequencing Panel and our NBIA Deletion/Duplication Panel include analysis of all 10 genes listed below.

<table>
<thead>
<tr>
<th>Gene / Condition</th>
<th>Clinical and Molecular Findings</th>
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<tbody>
<tr>
<td>ATP13A2</td>
<td>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 oculogryic 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).</td>
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<td>C19orf12</td>
<td>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).</td>
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<td>COASY</td>
<td>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.</td>
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<td>CP</td>
<td>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 dysartria (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]).</td>
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<td>DCAF17</td>
<td>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).</td>
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**FA2H**
FAHN (Fatty Acid Hydroxylase-Associated Neurodegeneration)

FA2H 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 opto atrophy and ocular motor abnormalities during the early stages of disease, and progressive intellectual impairment and seizures in the later stages. Iron accumulation occurs in the globus 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 glosis, 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. 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.
NBIA Sequencing Panel (10 genes: ATP13A2, C19orf12, COASY, CP, DCAF17, FA2H, FTL, PANK2, PLA2G6 and WDR45 sequencing)

Sample specifications: 3 to 10 cc of blood in a purple top (EDTA) tube
Cost: $2,500
CPT codes: 81407
Turn-around time: 8 weeks

Note: We cannot bill insurance for the above test.

NBIA Deletion/Duplication Panel (10 genes: ATP13A2, C19orf12, COASY, CP, DCAF17, FA2H, FTL, PANK2, PLA2G6 and WDR45 sequencing)

Sample specifications: 3 to 10 cc of blood in a purple top (EDTA) tube
Cost: $1,545
CPT codes: 81407
Turn-around time: 4-6 weeks

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

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: