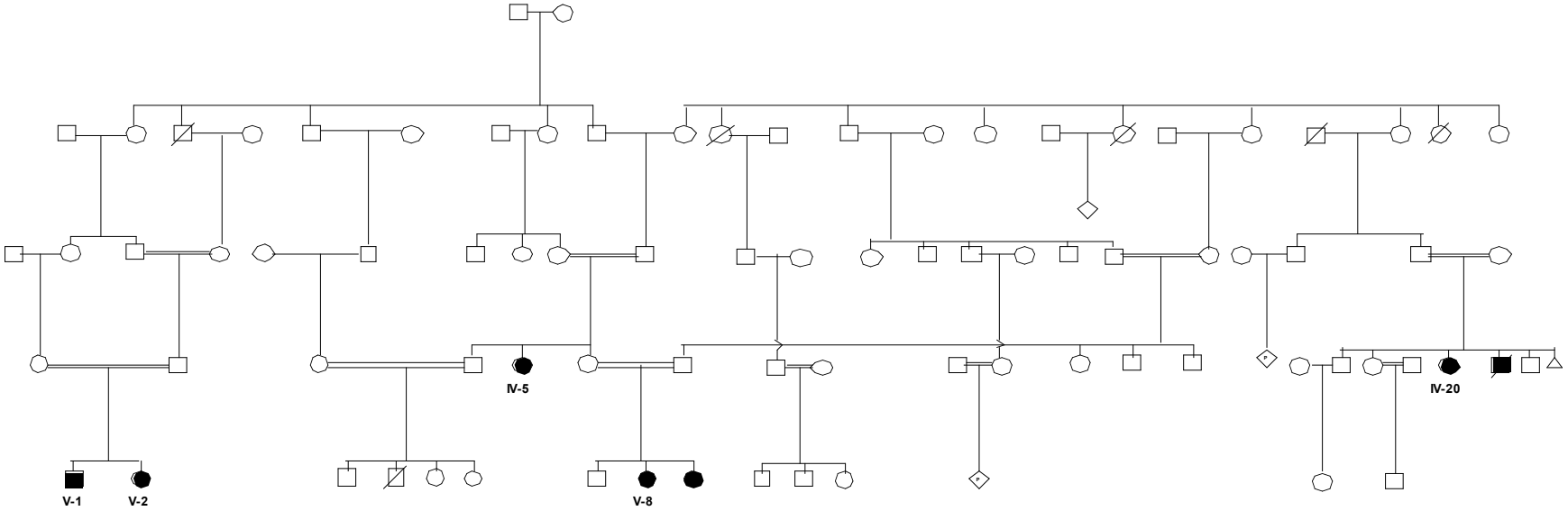
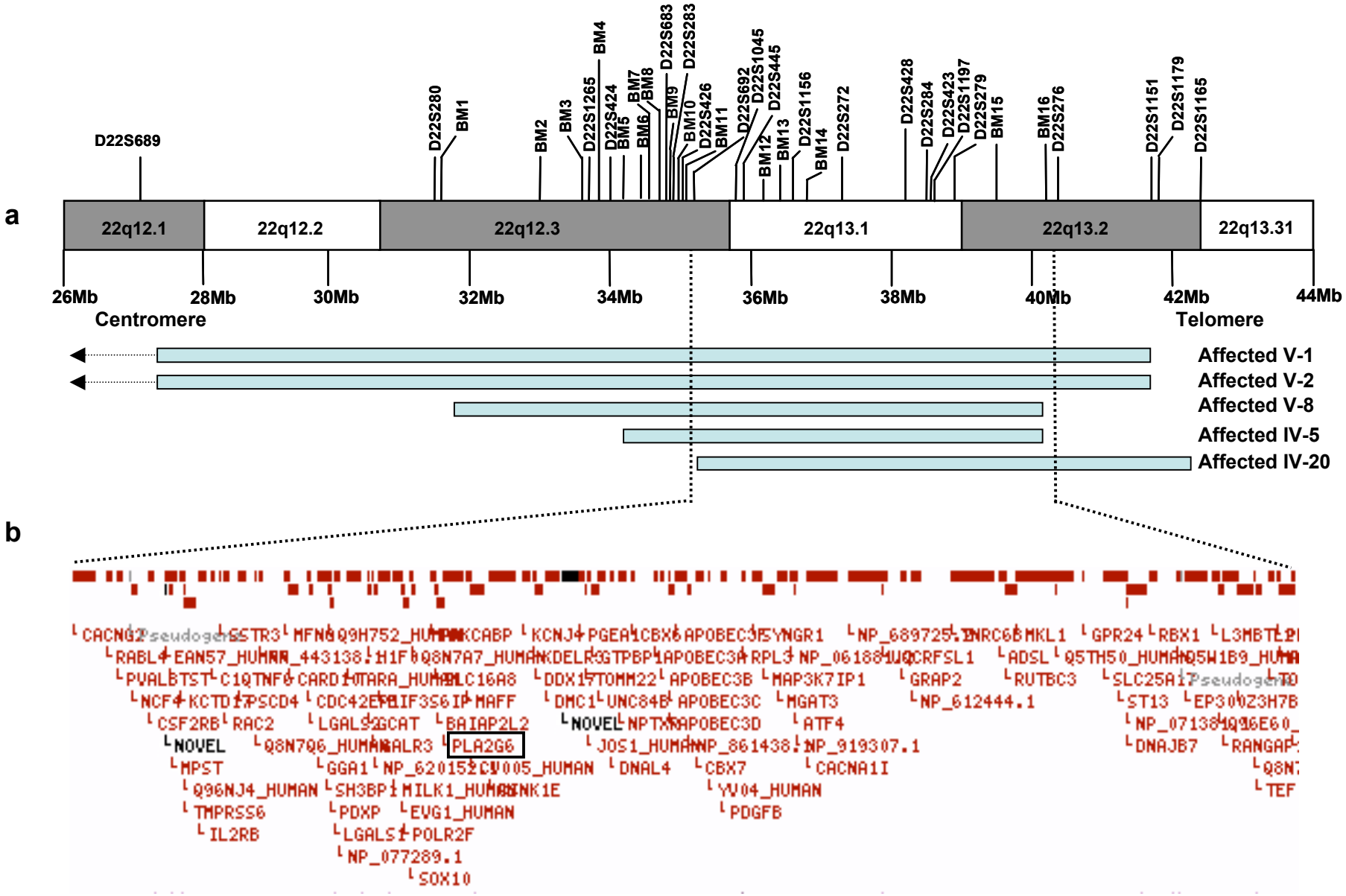
Supplementary Figure 1. Pedigree and genotypes of NBIA index family.



Marker	Decode cM	Marshfield cM	V-1		V-2		V-8		IV-5		IV-20	
D22S426		41.42	220	220	220	220	220	220	220	220	220	220
BM11			186	186	186	186	186	186	186	186	186	188
D22S692	42.27	41.42	156	156	156	156	156	156	156	156	156	156
D22S1045	44.75	42.81	139	139	139	139	139	139	139	139	139	139
D22S445	45.22	45.82	118	118	118	118	118	118	118	118	118	118
BM12			362	362	362	362	362	362	356	362	362	362
BM13			127	127	127	127	127	127	127	127	127	127
D22S1156	46.24	44.32	148	148	148	148	148	148	154	148	148	148
BM14			267	267	267	267	267	267	271	267	267	267
D22S272	47.33		131	131	131	131	131	131	131	139	131	131
D22S428	48.52	46.42	144	144	144	144	144	144	148	144	144	144
D22S284	49.01		81	81	81	81	81	81	97	81	81	81
D22S423	49.14	46.42	310	310	310	310	310	310	306	310	310	310
D22S1197					167	167	167	167	167	167	167	167
D22S279			173	173	173	173	173	173	173	173	173	173
BM15			221	221	221	221	221	221	215	221	221	221
BM16			169	169	169	169	169	169	179	169	169	169
D22S276	49.28	47.31	244	244	244	244	240	244	240	252	244	244

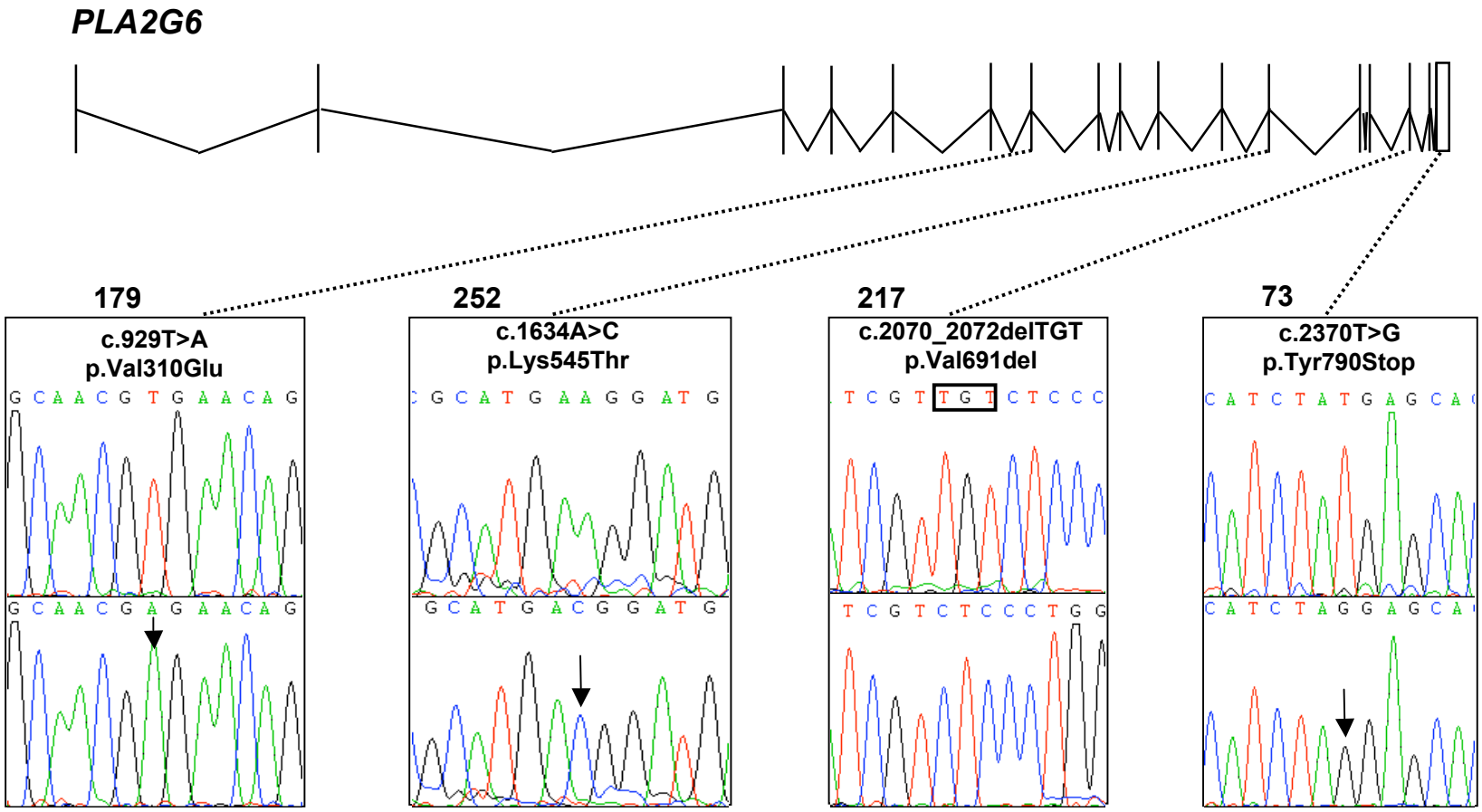
Legend. Pedigree is shown for the large consanguineous Pakistani family (252) with NBIA. Genotypes are for markers from chromosome 22q12-13 at the linked *INAD1* locus. The genetic distance of each marker is taken from the high resolution deCODE and Marshfield genetic maps and listed on the left. Shaded regions indicate the disease haplotype. The minimal candidate interval that encompasses the disease locus is the region between markers *D22S426* and *D22S276*.

Supplementary Figure 2. *INAD1* locus candidate region.



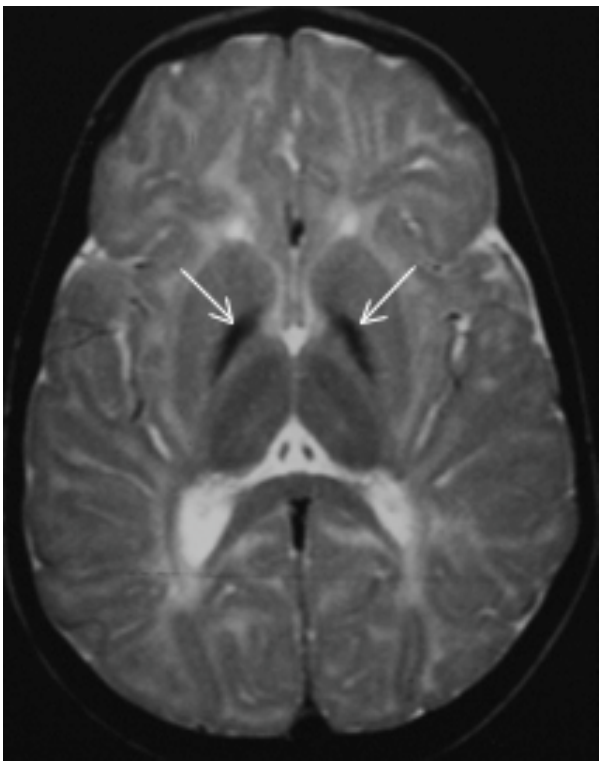
Legend. a) Schematic of INAD and NBIA linkage region on chromosome 22q12.3-q13.2 showing overlapping homozygous regions in 5 affected individuals of family 252, which delineates the candidate region; b) Positions of candidate genes taken from the Ensembl genome browser (Build v35.1). *PLA2G6* is shown in the black box.

Supplementary Figure 3. *PLA2G6* genomic organization and index patient mutations.



Legend. Exon organization of *PLA2G6* and positions of mutations found in the index NBIA and INAD families. Patient numbers according to Table 1 in the text are shown above the sequence traces.

Supplementary Figure 4. NBIA patient brain MRI.



Legend. T2-weighted brain MRI showing isolated hypointense signal in the globus pallidus (arrows), which is characteristic of non-PKAN NBIA and is seen in many of the INAD cases reported herein. The appearance is distinct from the eye-of-the-tiger sign seen in PKAN.

Supplementary Table 1. *PLA2G6* PCR and Sequencing Primers

Exon	Primer	Sequence
1	F	gacagggccaccagtgattg
	R	agttcgagatgagacacgggc
2	F	caggatctggggacaacgc
	R	gccaataagacctccaatcc
3	F	gggaccttctgattccagc
	R	gcccacacaagcaggtacac
4	F	aaagtccgagtttccgagtg
	R	aggcctgagagtgacacctg
5	F	cccggcctctttacgttc
	R	ctcaggcacgggacagg
6	F	cttcatcccacgccacg
	R	gaacctgcttcctgaggg
7	F	cagtgcccacgtgtccc
	R	gacagccctcctgcattc
8	F	ctttgttcttcacttccccg
	R	ctcggtccctgtatccacc
9	F	agctgcttgggatgtaccagc
	R	cggcttcctttagtgacttccg
10	F	ctagggacctctggggtagc
	R	gtgaggggcaggaaagc
11	F	aaagtactgggctgtggcag
	R	gcaaagccctgaagacaaac
12	F	aatttgggtttgcttaggcctc
	R	gttccctctgctcccctcaag
13	F	aattgtggggaaagggaaag
	R	accaccccacagcctctc
14	F	catgggttttatgccagtcc
	R	gtccctagcatggtttgctg
15	F	ccccagagcccagtcttg
	R	gtctcctccaacaccaaagg
16+17	F	gctccgagagtgcaggg
	R	gcaggggctgaatggac

Supplementary Methods

Clinical delineation: All human studies were performed using protocols and consent forms approved by the UB, OHSU or UCSF Institutional Review Boards or South Birmingham Ethics Committee. Patients were diagnosed with INAD based on clinical and family history, radiographic studies, and neuropathologic analysis of skin, conjunctiva, or sural nerve. For additional information see Supplementary Clinical Note.

Patient samples: DNA was isolated from whole blood. Lymphoblast or fibroblast cell lines were established by EBV transformation or standard methods. DNA was isolated from cell lines using the PUREGENE DNA Isolation Kit according to the manufacturer's instructions (Gentra Systems, Minneapolis, MN).

Genetic linkage studies: The genome-wide linkage screen of 31 INAD families and the 252 family were undertaken by the Center for Inherited Disease Research and the MRC Geneservice using a set of 400 short tandem repeat polymorphisms. Data from all chromosomes were analyzed by pairwise linkage analysis in GENEHUNTER v2.1_r5[1] (credit for the Mac OS X GENEHUNTER port: Rhett Sutphin at U. Iowa) or manually searching for extended regions of homozygosity. Regions showing positive LOD scores underwent further multipoint analysis using an autosomal recessive, fully penetrant model. Detailed mapping of the INAD and 252 families was performed with 49 chromosome 22 microsatellite markers: *D22S420, D22S345, D22S1167, D22S689, D22S1150, D22S1176, D22S280, D22S1265, D22S424, D22S685, D22S691, D22S683, D22S283, D22S426, D22S692, D22S1177, D22S1045, D22S445, D22S1156, D22S272, D22S428, D22S284,D22S423, D22S1197, D22S279, D22S276, D22S1151, D22S1179, D22S1165, D22S1169, D22S1171, D22S1168, D22S274* and 16 novel markers (BM 1-16; see Supplementary Figure 1). HEX or FAM fluorescently labeled M13 primers were obtained from Invitrogen. Marker-specific forward primers contained STRP flanking sequence preceded by the M13 primer sequence; reverse primers contained a 5'-antistutter sequence GTGTCTT. Initial PCR amplification was performed using touchdown PCR on an ABI 9700 thermocycler. Analysis of PCR products was performed using an ABI Prism 3100 genetic analyzer. Sizing was determined using ABI GeneScan-500 ROX standards and analysis was by

ABI GeneScan software followed by manual scoring by personnel blinded to the sample identities. Single nucleotide polymorphisms approximately 1 Mbp apart were selected on the basis of high heterozygosity (≥45%) and were sequenced.

Mutation analysis: We identified positional candidate genes using data from the National Center for Biotechnology Information (NCBI) and University of California Santa Cruz human genome databases. Sequencing was performed using standard methods[2]. For *PLA2G6*, we designed primer sets to amplify exons 1-15 and 16/17 and adjacent intronic sequence including splice signals (see Supplementary Table 1). Sequences were evaluated using BLAST at the NCBI, and traces were viewed with FinchTV (http://www.geospiza.com/).

Accession numbers: Amino acid and nucleotide numbering are for *PLA2G6*, variant 1 (also known as isoform a, or the long form) and correspond to NCBI reference sequence accession number NM_003560 for the cDNA and NP_003551 for the protein. Nucleotides were numbered using position 137 of NM_003560 as the +1 nucleotide of the coding sequence.

References

1. Kruglyak, L., Daly, M.J. & Lander, E.S. Rapid multipoint linkage analysis of recessive traits in nuclear families, including homozygosity mapping. *Am J Hum Genet* **56**, 519-27 (1995).
2. Hayflick, S.J. et al. Genetic, clinical, and radiographic delineation of Hallervorden-Spatz syndrome. *N Engl J Med* **348**, 33-40 (2003).

Supplementary Clinical Note

Human research subjects were recruited as part of an OHSU IRB-approved protocol that includes maintaining an international INAD family registry with collection of blood samples and clinical information. Participants included in the study were referred to our registry by their neurologists or other specialists or contacted us directly.

The original 12 families utilized for linkage studies all contained probands meeting the diagnostic criteria for classic INAD as established at the First Scientific Meeting on Infantile Neuroaxonal Dystrophy in 2002 (http://www.orpha.net/associations/DNAI/cgi-bin/DNAI/index.php?lng=en). These criteria are as follows:

Classic INAD

Obligatory

-Onset < 3 years
-Clinical evidence for central nervous system involvement
-Histopathologic evidence of dystrophic axons on biopsy from one or more of the following tissues: conjunctiva, skin, muscle, peripheral nerve (sural), or rectum.
  -Membranotubular profiles
  -Mitochondrial aggregates
  -Axons with increased diameter and thinned membrane
-Psychomotor regression
-Progression

Corroborative

-Cerebellar atrophy
-Optic atrophy
-Axial hypotonia leading to spasticity and rigidity
-Symmetric pyramidal tract signs
-Seizures
-Nystagmus
-Strabismus
-EMG => denervation
-EEG => fast rhythms
-VEP => delayed with reduced amplitudes
-Hypointense globus pallidus on T2-weighted brain MRI

Exclusionary

-Central hyperintensity within a region of hypointensity in the medial globus pallidus (eye-of-the-tiger sign) on T2-weighted brain MRI
-*PANK2* mutation
-NAGA deficiency
-Ceroid lipofuscin accumulation on biopsy
-Congenital ataxia
-Primary movement disorder
-Copper metabolic abnormality

The three INAD individuals used for sequencing candidate genes met these clinical criteria. They were also selected based on strong linkage to the candidate region. To investigate the molecular basis of NBIA, family 252 was used for linkage studies and candidate gene screening. Affected individuals in this family have abnormal iron accumulation in the globus pallidus demonstrated by T2-weighted MRI, and *PANK2* mutations were ruled out.