



Published in final edited form as:

*Nat Genet.* 2006 July ; 38(7): 752–754.

## **PLA2G6, encoding a phospholipase A<sub>2</sub>, is mutated in neurodegenerative disorders with high brain iron**

Neil V Morgan<sup>1,18</sup>, Shawn K Westaway<sup>2,18</sup>, Jenny E V Morton<sup>3</sup>, Allison Gregory<sup>2</sup>, Paul Gissen<sup>1</sup>, Scott Sonek<sup>2</sup>, Hakan Cangul<sup>1,4</sup>, Jason Coryell<sup>2</sup>, Natalie Canham<sup>3</sup>, Nardo Nardocci<sup>5</sup>, Giovanna Zorzi<sup>5</sup>, Shanaz Pasha<sup>1</sup>, Diana Rodriguez<sup>6</sup>, Isabelle Desguerre<sup>7</sup>, Amar Mubaidin<sup>8</sup>, Enrico Bertini<sup>9</sup>, Richard C Trembath<sup>10</sup>, Alessandro Simonati<sup>11</sup>, Carolyn Schanen<sup>12</sup>, Colin A Johnson<sup>1</sup>, Barbara Levinson<sup>13</sup>, C Geoffrey Woods<sup>14</sup>, Beth Wilmot<sup>2</sup>, Patricia Kramer<sup>2,15</sup>, Jane Gitschier<sup>13,16</sup>, Eamonn R Maher<sup>1,3</sup>, and Susan J Hayflick<sup>2,15,17</sup>

1 Section of Medical & Molecular Genetics, University of Birmingham School of Medicine, Edgbaston, Birmingham B15 2TT, UK

2 Department of Molecular and Medical Genetics, Oregon Health & Science University, Portland, Oregon, USA 97239

3 West Midlands Regional Genetics Service, Birmingham Women's Hospital, Birmingham, UK B15 2TG

4 Department of Medical Genetics, Uludag University School of Medicine, Bursa 16036, Turkey

5 Department of Child Neurology, Istituto Nazionale Neurologico "Carlo Besta", Milan 20133, Italy

6 Department of Pediatric Neurology, Hôpital Armand Trousseau, Paris 75571, France

7 Department of Pediatric Neurology, Hôpital Necker Enfants Malades, Paris 75270, France

8 Neurology Department, King Hussein Medical Centre, Amman 11947, Jordan

9 Unit of Molecular Medicine, Bambino Gesù Hospital, Rome 00165, Italy

10 Division of Genetics and Molecular Medicine, Kings College London School of Medicine, Guys Hospital, London SE1 9RT, UK

11 Department of Neurological and Visual Sciences, University of Verona School of Medicine, Verona 37129, Italy

12 Nemours Research Programs, A.I. duPont Hospital for Children, Wilmington, Delaware 19899, USA

13 Department of Medicine, University of California, San Francisco, California 94143, USA

14 Department of Medical Genetics, Cambridge Institute for Medical Research, University of Cambridge, Cambridge CB2 1TN, UK

15 Department of Neurology, Oregon Health & Science University, Portland, Oregon 97239, USA

16 Howard Hughes Medical Institute.

17 Department of Pediatrics, Oregon Health & Science University, Portland, Oregon 97239, USA

### **Abstract**

Correspondence should be addressed to S.J.H. (hayflick@ohsu.edu).

<sup>18</sup>These individuals contributed equally to this work.

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#### COMPETING INTERESTS STATEMENT

The authors declare that they have no competing interests.

Note: Supplementary information is available on the Nature Genetics website.

Neurodegenerative disorders with high brain iron include Parkinson disease, Alzheimer disease and several childhood genetic disorders categorized as neuroaxonal dystrophies. We mapped a locus for infantile neuroaxonal dystrophy (INAD) and neurodegeneration with brain iron accumulation (NBIA) to chromosome 22q12-q13 and identified mutations in *PLA2G6*, encoding a calcium-independent group VI phospholipase A<sub>2</sub>, in NBIA, INAD and the related Karak syndrome. This discovery implicates phospholipases in the pathogenesis of neurodegenerative disorders with iron dyshomeostasis.

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The neuroaxonal dystrophies include INAD, NBIA and Schindler disease and share the distinctive pathologic feature of axonal degeneration with distended axons (spheroid bodies) throughout the central nervous system. INAD is characterized by progressive motor and sensory impairment, with spheroids also found in peripheral nerves<sup>1</sup> (Supplementary Note online). NBIA comprises a clinically and genetically heterogeneous group of disorders with high basal ganglia iron and includes pantothenate kinase-associated neurodegeneration caused by mutations in *PANK2* (refs. 2-3). Some individuals with INAD show high brain iron; hence, these phenotypes do overlap. To investigate the molecular bases of these two forms of neuroaxonal dystrophy, we undertook genome-wide linkage studies in 12 families with INAD and in a large consanguineous Pakistani family with NBIA (family 252). Using polymorphic microsatellite markers, we mapped a novel *INADI* locus to a 6.0-Mb region of 22q12.3-q13.2 (LOD score of 4.78 at *D22S692*), with evidence of locus heterogeneity (Supplementary Methods online). We also detected linkage to chromosome 22q12-q13 in family 252 with NBIA (LOD score 4.65), with a minimal region of linkage of 4.9 Mb between *D22S426* and *D22S276* containing the disease locus (Supplementary Fig. 1 and Supplementary Fig. 2 online).

In view of the common regions of linkage, we predicted that the NBIA variant in family 252 and INAD would be allelic and proceeded to sequence candidate genes in DNA from family 252 and three INAD probands. After sequencing 70 of ~100 positional candidate genes, we detected mutations in *PLA2G6* in all four kindreds (Table 1 and Supplementary Fig. 3 online). We detected a homozygous missense mutation (leading to the amino acid change L545T) in the proband of family 252. This mutation segregated with disease status in 15 affected and unaffected family members. We did not detect this change in 192 ethnically matched control chromosomes. We also detected *PLA2G6* mutations in the three INAD probands. We did not detect any of these changes in 192 control chromosomes. We also identified *PLA2G6* mutations in 28 additional probands with INAD and in the Karak syndrome family with high basal ganglia iron reported previously<sup>4</sup>. We identified a total of 44 unique mutations (32 missense, five deletions leading to a frame-shift, three nonsense, two leading to amino acid deletions without a frameshift, one splice site and one large deletion) (Table 1 and Fig. 1). Of the missense mutations, 85% occurred at amino acid positions that are conserved in vertebrates.

We reviewed the clinical and brain magnetic resonance imaging (MRI) data from individuals with *PLA2G6* mutations (Supplementary Fig. 4 online). Although brain MRI changes indicating high levels of iron have been reported only rarely in INAD<sup>5,6</sup>, 8 of 20 kindreds (40%) with mutation-positive INAD showed high iron in the globus pallidus. This high frequency may be skewed by a bias in our collection toward those with high brain iron; nevertheless, INAD should clearly be included in the differential diagnosis of neurodegeneration with high brain iron. In contrast, we identified *PLA2G6* mutations in only 4 of 24 additional individuals diagnosed with NBIA. *PLA2G6* genotype correlates with phenotype only insofar as we found homozygous null mutations only in INAD and not in any individuals with a clinical diagnosis of NBIA.

Historically, diagnosis of INAD has required histopathological evidence of dystrophic axons. However, we found that the presence of spheroids on biopsy did not absolutely correlate with mutations in *PLA2G6*. Five individuals with clinical and pathological features of INAD were

negative for *PLA2G6* mutations. Incomplete detection of mutations could explain this; however, we were able to identify both mutated alleles in all but one individual with mutations. An alternative explanation is that INAD is genetically heterogeneous. Indeed, although *PLA2G6* is the major disease locus, our linkage data support the existence of at least one additional INAD locus (data not shown).

Although spheroids are characteristic of INAD, three individuals with a clinical diagnosis of INAD but without spheroids were found to have mutations in *PLA2G6*. Possible reasons for normal pathologic studies include biopsy at an age before pathological changes have developed or from a site where changes are not evident. Although individual 256 had only a single negative biopsy at age 2 years, individuals 16 and 172-2 (ref. 1) had negative biopsies at 12 years of age. The latter's sibling (172-1) was positive both for mutations in *PLA2G6* and for spheroids (at age 11 years)<sup>1</sup>. These observations indicate a role for *PLA2G6* mutation detection in the diagnostic evaluation of all individuals suspected to have INAD, regardless of biopsy results. Indeed, we would argue that molecular testing should replace this more invasive procedure and that *PLA2G6* mutation studies are indicated for individuals with a phenotype in the spectrum of NBIA as well.

*PLA2G6* encodes iPLA<sub>2</sub>-VI, a calcium-independent phospholipase. Phospholipase A<sub>2</sub> enzymes catalyze the hydrolysis of glycerophospholipids at the *sn*-2 position, generating a free fatty acid, usually arachidonic acid, and a lysophospholipid. The iPLA<sub>2</sub>-VI protein is active as a tetramer, with proposed roles in phospholipid remodeling, arachidonic acid release, leukotriene and prostaglandin synthesis and apoptosis<sup>7</sup>. *PLA2G6* transcript variants encode multiple isoforms<sup>8</sup>, and the two enzymatically active forms are predicted to be affected by all of the mutations reported here. A subset of mutations would also alter the shorter enzymatically inactive isoforms, which seem to act as dominant-negative inhibitors when incorporated into the tetramer<sup>7,8</sup>. Because all of the mutations identified are likely to be inactivating, no additional effect is predicted for this subset, and indeed, no distinctive phenotype is recognized.

The calcium-independent PLA<sub>2</sub> (iPLA<sub>2</sub>) enzymes are critical in cell membrane homeostasis. Levels of phosphatidylcholine, abundant in mammalian cell membranes and key in maintaining membrane integrity, are regulated by the opposing actions of CTP:cytidylphosphocholine transferase and iPLA<sub>2</sub>-VIA<sup>9</sup>. Defects in iPLA<sub>2</sub>-VIA could lead to a relative abundance of membrane phosphatidylcholine and secondary structural abnormalities, which may underlie the axonal pathology observed in INAD and NBIA.

Although defective phospholipid metabolism has been suspected in neurodegeneration<sup>9,10</sup>, our discovery clearly implicates phospholipase A<sub>2</sub> dysfunction in neurodegeneration and more specifically in disease associated with brain iron dyshomeostasis. Impaired phospholipid metabolism has also been implicated in the pathogenesis of another form of NBIA, pantothenate kinase-associated neurodegeneration<sup>3</sup>. Iron accumulates with age in brain regions that are targeted in Alzheimer disease and Parkinson disease, and iron is implicated in their pathogenesis<sup>11</sup>. Hence, this new link between phospholipid defects and brain iron metabolism may generate insights into the pathogenesis of common neurodegenerative disorders and may spur ideas for new neuroprotective strategies.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

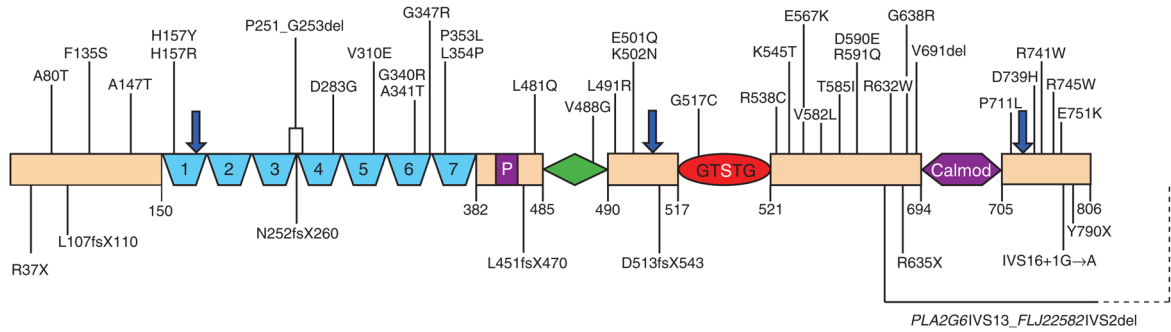
### Acknowledgements

We are grateful to the many contributing individuals, families and physicians. This work was funded by the Wellcome Trust and WellChild (E.M.), by the US National Eye Institute, L'Association Internationale De Dystrophie Neuro Axonale Infantile, the US National Institute of Child Health and Human Development, and the R.J. Murdock

Foundation (S.J.H.) and by the NBIA Disorders Association and NORD (E.M., S.J.H.). Additional support was provided by the Paolo Zorzi Foundation, the Italian National Ministry of Health and the Oregon Alzheimer Disease Center Grant (P30 AG008017). Genotyping services were provided by the CIDR through a federal contract from the US NIH to The Johns Hopkins University, contract number N01-HG-65403 and by the OHSU GCRC (grant M01 RR000334). We thank H. Consenego for technical assistance. J.G. is an investigator with the Howard Hughes Medical Institute.

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**Figure 1.**

Schematic showing iPLA<sub>2</sub>-VIA protein structure<sup>7</sup> and mutation-derived changes. Variant 1 is shown with seven ankyrin repeats (blue), a proline-rich motif (purple box), a glycine-rich nucleotide binding motif (green diamond), a lipase motif (red with the active site, Ser519, highlighted), a proposed C-terminal Ca<sup>2+</sup>-dependent calmodulin binding domain (purple) and three putative caspase cleavage sites (blue arrows). Numbers indicate amino acids.

Substitutions or deletions of amino acids not altering the reading frame are shown above the diagram; nonsense mutations, splice mutations and deletions causing frameshifts are shown below. A deletion encompassing exons 14 through 17, the 3' of *PLA2G6* and exons 1 and 2 of a downstream putative transcript, *FLJ22582*, is shown at the C terminus.

Table 1

Phenotypically classified individuals and their mutations

Individual	Exon	Mutation	Result	Spheroids	Brain iron
NBIA with PLA2G6 mutations					
45	3	238G→A	A80T	u	+
	17	2370_2371delTTG	Y790X		
102	3	319delC	L107fsX110	No biopsy	+
125	16	2251G→A <sup>b</sup>	E751K	No biopsy	+
252 <sup>ac</sup>	12	1634A→C <sup>b</sup>	K545T	No biopsy	+
286 <sup>d</sup>	14	1894C→T	R632W	No biopsy	+
	15	2070_2072delTTG	V691del		
288	7	1061T→C	L354P	No biopsy	+
	16	2215G→C	D739H		
Classical INAD					
73 <sup>a</sup>	17	2370T→G <sup>b</sup>	Y790X	+	-
81	3	404T→C	F135S	+	-
	11	1472T→G	L491R		
144 <sup>a</sup>	14;17	PLA2G6IVS13_FLJ225821VS2del <sup>b</sup>	Fusion protein	+	u
173 <sup>d</sup>	2	109C→T	R37X	+	-
	11	1501G→C	E501Q		
177 <sup>a</sup>	13	1772G→A <sup>b</sup>	R591Q	+	-
182 <sup>a</sup>	2	109C→T <sup>b</sup>	R37X	+	-
184 <sup>a</sup>	7	1018G→A <sup>b</sup>	G340R	+	u
186	11	1538_1541delACTG	D513fsX543	+	-
211-1 <sup>a</sup>	17	2370_2371delTTG	Y790X	+	u
212	10	1351delC <sup>b</sup>	L451fsX470	+	-
	14	1912G>A	G638R	+	-
	16	2221C→T	R741W		
229	11	1506G→C	K502N	+	-
	12	1612C→T	R538C		
231	7	1039G→A <sup>b</sup>	G347R	+	-
277	16	IVS16+1G→A <sup>b</sup>	Aberrant splicing	No biopsy	-
INAD with brain iron					
16	13	1744G→T	V582L	-	+
	16	2233C→T	R745W		
171 <sup>a</sup>	4	469C→T <sup>b</sup>	H157Y	+	+
172-1 <sup>e</sup>	4	470A→G	H157R	+	+
	7	1021G→A	A341T		
172-2 <sup>f</sup>	4	470A→G	H157R	-	+
	7	1021G→A	A341T		
176 <sup>a</sup>	2	109C→T <sup>b</sup>	R37X	+	+
180	13	1770C→A	D590E	+	+
	16	2221C→T	R741W		
211-2 <sup>a</sup>	10	1351delC <sup>b</sup>	L451fsX470	U	+
256	5	755delA	N252fsX260	-	+
	7	1061T→C	L354P		
273	12	1699G→A	E567K	+	+
	13	1754C→T	T585I	+	+

Individual	Exon	Mutation	Result	Spheroids	Brain iron
Classical INAD by report					
178	6	848A→G <sup>b</sup>	D283G		
179	7	929T→A <sup>b</sup>	V310E		
188	4	439G→A	A147T		
	15	2132C→T	P711L		
189	11	1442T→A <sup>b</sup>	L481Q		
190	4	470A→G	H157R		
	14	1903C→T	R635X		
191	14	1903C→T <sup>b</sup>	R635X		
192	5	751_759del9bp	P251_G253del		
	11	1463T→G	V488G		
193	11	1442T→A <sup>b</sup>	L481Q		
194	14:17	PLA2G6IVS13_FLJ22582IVS2del <sup>b</sup>	Fusion protein		
217	15	2070_2072delTTG <sup>b</sup>	V691del		
264	7	1058C→T	P353L		
	11	1549G→T	G517C		

Survey of a large number of ESTs in the public database did not uncover any of the sequence changes described here. X indicates stop. + = present, - = absent, u = unknown. See Supplementary Table 1 online for primer sequences. Written informed consent was obtained from all study participants. This study was approved by institutional review boards of Oregon Health & Science University or the University of Birmingham

<sup>a</sup>Parents are known to be consanguineous, or there is probable identity by descent based on haplotypes.

<sup>b</sup>Mutation is homozygous. Individuals who are unlikely to be identical by descent and who seem to be homozygous for one mutation by sequence analysis may be hemizygous because of a deletion of one allele.

<sup>c</sup>Individual from index NBIA family.

<sup>d,e,f</sup>Three individuals were previously reported in ref. 1.