Published in final edited form as:

Brain Res Bull. 2010 November 20; 83(6): 374–379. doi:10.1016/j.brainresbull.2010.08.011.

Expression of *PLA2G6* in human fetal development: Implications for infantile neuroaxonal dystrophy

Brenda Polster^{1,*}, Moira Crosier², Susan Lindsay², and Susan Hayflick¹

¹Molecular and Medical Genetics, Oregon Health & Science University, Portland OR

²MRC-Wellcome Trust Human Developmental Biology Resource, Institute of Human Genetics, Newcastle University, UK, NE1 3BZ

Abstract

Mutations in *PLA2G6*, which encodes calcium-independent phospholipase A₂ group VIA (iPLA₂-VIA), underlie the autosomal recessive disorder infantile neuroaxonal dystrophy (INAD). INAD typically presents in the first year of life, and leads to optic atrophy and psychomotor regression. We have examined *PLA2G6* expression in early human embryonic development by *in situ* hybridization. At Carnegie Stage (CS) 19 (approximately 7 post conception weeks [PCW]), strong expression is evident in the ventricular zone (VZ) of midbrain and forebrain suggestive of expression in neural stem and progenitor cells. At CS23 (8 PCW) expression is also detectable in the VZ of the hindbrain and the subventricular zone (SVZ) of the developing neocortex, ganglionic eminences and diencephalon. By 9 PCW strong expression in the post-mitotic cells of the cortical plate can be seen in the developing neocortex. In the eye, expression is seen in the lens and retina at all stages examined. *PLA2G6* expression is also evident in the alar plate of the spinal cord, dorsal root ganglia, the retina and lens in the eye and several non-neuronal tissues, including developing bones, lung, kidney and gut. These findings suggest a role for *PLA2G6* in neuronal proliferation throughout the developing brain and in maturing neurons in the cortical plate and hindbrain. Although widespread *PLA2G6* expression is detected in neuronal tissues, the pattern shows dynamic changes with time and indicates that INAD pathogenesis may begin prior to birth.

Keywords

PLA2G6; INAD; neurodegeneration; development; in-situ hybridization

1. INTRODUCTION

Infantile neuroaxonal dystrophy (INAD) is an autosomal recessive disease with early onset and rapid progression of hypotonia, hyperreflexia and tetraparesis. INAD belongs to a larger class of neuroaxonal dystrophies, which include pantothenate-kinase associated neurodegeneration (PKAN), idiopathic neurodegeneration with brain iron accumulation (NBIA), and Schindler disease. Within this group, INAD has the earliest onset, with motor and cognitive skill regression presenting at a median age of 15 months [14,18]. Optic

© 2010 Elsevier Inc. All rights reserved.

*corresponding author, Phone: 503 494 2449, Fax: 503 494 6886, polsterb@ohsu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

atrophy is also commonly observed. Historically, INAD was diagnosed from histological evidence of neuroaxonal spheroids in peripheral nerves [24]. The detection of cerebellar atrophy and iron accumulation in the globus pallidus by MRI is also diagnostic of INAD, although iron accumulation often is not observed until later in the disease [14,18,23].

In 2006, the causative gene for INAD was identified as *PLA2G6*, which encodes a calcium-independent phospholipase (A_2 group IVA) [23]. *PLA2G6* mutations are found in the vast majority of INAD patients and have also been described in individuals previously diagnosed with idiopathic NBIA [14], as well as individuals with adult-onset dystonia-parkinsonism [29]. Phospholipases A_2 comprise a large family of enzymes that catalyze the hydrolysis of sn-2 ester bonds of glycerophospholipids, producing free fatty acids and lysophospholipids [12]. Arachidonic acid and other fatty acids released by iPLA $_2$ -VIA can initiate apoptosis, inflammation, and cell growth [4,36]. The lysophospholipid remaining in the cell membrane can also trigger cellular processes, including chemotaxis and fusion of biological membranes [2,10].

The early presentation of INAD suggests that iPLA $_2$ -VIA may have a developmental role. Malik and colleagues [20] showed that the *Pla2g6* knockout mouse [5] develops neurological impairments by 13 months and accumulates neuroaxonal spheroids similar to those in human INAD patients. In addition, four month old *Pla2g6* knockout mice have decreased brain docosahexaenoic acid (DHA) metabolism and signaling [6]. In a second INAD mouse model, point mutations within *Pla2g6* result in motor dysfunction and neuroaxonal spheroids as early as 7 weeks of age [39]. These studies, however, did not examine *Pla2g6* expression during mouse development. *Pla2g6* expression has been shown in mouse sagittal sections at embryonic day 14.5 as part of the high-throughput Eurexpress project [38]. At this stage, the strongest expression seen in the brain is in the alar plate of the developing hindbrain with prominent expression also in an analogous region of the midbrain. *Pla2g6* also appears to be expressed weakly in the developing diencephalon and telencephalon of the forebrain. Expression was also detected in spinal cord and the bones of the developing skull, face, ribcage and limbs (EMAGE entry 18166; genex.hgu.mrc.ac.uk).

To better understand the potential role for *PLA2G6* in neuronal development and pathogenic mechanisms underlying disease, we examined *PLA2G6* expression by *in-situ* hybridization across several stages of human embryonic and early fetal development.

2. MATERIALS AND METHODS

2.1 Human tissue collection and processing

Human embryonic and fetal tissues were obtained from the MRC-Wellcome Trust Human Developmental Biology Resource (www.hdbr.org), Institute of Human Genetics, Newcastle University. The samples were collected with appropriate maternal consents and ethical approval by the Newcastle and North Tyneside Research Ethics Committee. Tissue sections from 7 samples were analysed: Carnegie Stage (CS) 19 (~7 PCW, n=2), CS23 (8PCW, n=3) and 9 PCW (n=2). The stage of embryonic development (CS19 and CS23) was determined by assessment of external morphology as described [9,25]. For fetal samples (9PCW) age was estimated from measurements of foot length and heel to knee length. These were compared with a standard growth chart [15].

2.2 In situ hybridization

Three fragments of the cDNA for human *PLA2G6*; 453 bp of *PLA2G6* exon 2- exon4 (probe 1), 517 bp of *PLA2G6* exon 11–15 (probe2) and 557 bp of the *PLA2G6* 3'UTR (probe3) were amplified from Homo sapiens *PLA2G6* mRNA (Accession: CU013143) and cloned into pCR-BluntII-TOPO (Invitrogen). The construct sequences were verified and prepared

using HiSpeed Plasmid Midi kit (Qiagen). To create the *PLA2G6* antisense probe, the plasmid was linearized with *SpeI* and amplified from the T7 promoter. To create the *PLA2G6* sense control probe, the plasmid was linearized with *NotI* and amplified from the *Sp6* promoter. Probes were labeled with digoxigenin (DIG) using the DIG-RNA labeling kit (Roche Applied Science) according to manufacturer's instructions. All probes were tested and *PLA2G6* probe 3 was selected for the studies subsequently described.

In situ hybridizations were performed as previously described [22] with some modifications. Briefly, sections were dewaxed in xylene, gradually hydrated in decreasing ethanol concentrations before incubation in Proteinase K (20 µg/ml) at room temperature (RT), followed by fixation in 4% paraformaldehyde in PBS. Background was reduced by treating with 0.1M Triethanolamine pH 8. Sections were air dried and probe added (300ng labeled probe per 100ul of Dig Easy Hyb mix (Roche)) at 68°C overnight. The next day sections were washed once in 5× SSC then once in 2× SSC at 60°C then incubated with Anti-digoxigenin AP Fab fragments (Roche) diluted 1:1000 at 4°C overnight. Sections were then washed and expression detected using NBT/BCIP (20ul/ml Roche) in 0.1M Tris/0.1M NaCl (pH 9.5) in the dark at RT. Developing was stopped by rinsing slides first in 0.1M Tris/0.1M NaCl (pH 9.5) then in distilled H₂O. Sections were mounted using Aquamount and analyzed using a Zeiss Axioplan 2 microscope. Images were captured with a Zeiss Axiovision 4 imaging system.

3. RESULTS

In order to characterize the potential role of *PLA2G6* in fetal development, we examined *PLA2G6* expression by *in situ* hybridization during human embryonic (CS19 and CS23) and early fetal (9 PCW) development.

3.1 *PLA2G6* expression in the brain

At CS19, *PLA2G6* expression was evident in the VZ of the pallium neuroepithelium (NEP) in the developing telencephalon (Figure 1A). The NEP is a pluripotent pseudostratified tissue of neural stem cells that extends from the frontal pole to the spinal cord and is the source of all neurons and neuroglia of the developing nervous system [7]. By CS23, *PLA2G6* expression is detected in both the VZ and SVZ of the pallium, while by 9PCW strong signal is also detected in the differentiating neurons of the cortical plate (Figure 1B and C respectively). Strong expression is also seen in proliferative zones in other regions of the developing forebrain such as the ganglionic eminences (Figure 1B, D, E) and thalamus (Figure 1D, F).

In the midbrain, *PLA2G6* expression is detectable in the VZ of the alar plate at CS19 (Figure 1J), CS23 (Figure 1H) and persists through to 9PCW (data not shown). In contrast, expression in the hindbrain is barely detectable at CS19 (data not shown). Yet at CS23 (Figure 1H and I) and 9PCW (Figure 1K and L), *PLA2G6* is expressed in the VZ in the isthmus, cerebellum, pons and medulla oblongata and clearly present in differentiating neurons of developing nuclei in the medulla (Fig 1K and L). *PLA2G6* expression is also seen in the telencephalic choroid plexus, a glycogen-rich epithelial tissue formed by proliferative stem cells that may play a part in anaerobic metabolism during early development [7] (data not shown).

3.2 *PLA2G6* expression in the eye and spinal cord

PLA2G6 expression was found in the lens and developing neurosensory retina at CS19, CS23 and PCW9 (Figure 2). Expression in the retina was sparse initially but became stronger and more widespread throughout the developing retina by 9PCW. Interestingly, in

one CS23 sample, we also observed strong staining in the optic nerve (Figure 2B). The optic nerve comprises axons from retinal ganglion cells and glial precursors: astrocyte precursors arising from the optic stalk and oligodendrocyte precursors from the preoptic area [27,35]. However, the optic nerve only begins to be myelinated at five months of gestation [13]. *PLA2G6* expression was detected in the spinal cord, principally in the alar plate, as well as in the dorsal root ganglia at CS19 and CS23. Spinal cord expression was not analyzed at 9PCW; however, we did observe it throughout the spinal cord and in dorsal root ganglia at 13PCW (data not shown).

3.3 PLA2G6 in non-neuronal tissues

Results from RT-PCR experiments in a battery of RNAs from different tissues at 9PCW suggested that *PLA2G6* was expressed widely in non-neuronal tissues (data not shown). This was confirmed by tissue *in situ* hybridisation as shown in Figure 3. *PLA2G6* expression was detected in lung (Figure 3A), kidney (both metanephros and mesonephros Figure 3B and C respectively), gonad (Figure 3C), muscles in the tongue and surrounding the eye (Figure 3D and 3H), nasal epithelium (Figure 3E), surrounding the developing bones (Figure 3F) and in the intestine (Figure 3G).

4. DISCUSSION

The present study was carried out to characterize *PLA2G6* expression in the developing human embryonic and fetal nervous system. We found that *PLA2G6* has a dynamic expression pattern both in terms of the location of expression and the differentiation state of expressing cells. *PLA2G6* is expressed in forebrain and midbrain before it is detectable in hindbrain. Throughout the developing brain, *PLA2G6* is expressed in proliferative zones: VZ in all regions, as well as the SVZ in the developing telencephalon including the ganglionic eminences. In the diencephalon there is strong expression in the thalamus and hypothalamus. This expression pattern is in contrast to the only available comparison data derived from studies in developing mouse; in murine studies VZ expression in the alar plate of the midbrain and hindbrain has been described, but none is seen in the VZ of the forebrain (diencephalon or telencephalon) and there is only weak expression in the outer cortical plate/mantle layer in the developing neocortex (EMAGE entry ID 18166). However, this mouse data is only from a single stage and does not capture the dynamic changes in expression pattern seen in human as described above. In our studies of the human forebrain, *PLA2G6* was also detected in the differentiated neurons of the cortical plate in the developing cerebral neocortex. Similarly, expression is seen in differentiated neurons in the hindbrain; for example, in the inferior olives of the medulla. At the stages examined, there is no *PLA2G6* expression in differentiated neurons of the midbrain.

Broad expression of iPLA₂ in the brain has also been reported in adult mammals. In adult monkey brain, high iPLA₂ expression was detected in the cerebral neocortex, hippocampus, cerebellum and brain stem, with lower expression in the thalamus and hypothalamus [26]. Within the basal ganglia, the caudate nucleus, putamen and nucleus accumbens were densely labeled while the globus pallidus, subthalamic nucleus and substantia nigra pars compacta were lightly labeled [26]. Within these tissues, iPLA₂ expression was predominately localized to the nuclear envelope of neurons, dendritic spines or axon terminals, while expression was sparse or absent in myelinated axons, large diameter dendrites, glial cells and endothelial cells [26]. iPLA₂ expression has also been reported in rat cerebellum, with strong expression in the nuclei of Purkinje cells and granule and stellate cells [34]. These findings are consistent with an ongoing role for iPLA₂ in signal transduction and lipid metabolism in a variety of CNS cell types. In the adult eye, iPLA₂-VIA expression has previously been reported as very strong in the retinal pigment epithelium, strong in the optic nerve axons and cornea, and moderate in the iris, ciliary body

and lens [17]. Interestingly, iPLA₂-VIA is proposed to regulate RPE proliferation and migration, and may play a role in proliferative vitreoretinopathy [16]. Our data indicates that *PLA2G6* is expressed in retina and lens as early as CS19 (approx 7 PCW). In addition, we observed expression in the sheath of the optic nerve in one sample. Although retinopathy is not common in *PLA2G6*-associated disease, optic atrophy is typical [14]. Indeed, Kurian et al. observed reduced volume in the optic chiasm and optic nerves on radiographical imaging in eight out of ten *PLA2G6* mutation-positive patients [18], consistent with an important postnatal role for *PLA2G6* in maintaining optic nerve integrity.

A survey of non-neuronal embryonic tissues revealed broad *PLA2G6* expression. Previously, *PLA2G6* was detected in all 23 adult human tissues examined by Northern blot [19]. In our study, strong expression was detected surrounding the developing bones of the arm and in the lung, kidney, gut and muscles of the tongue and surrounding the eye. At the stages examined, we did not detect strong expression in the developing bones of the face and skull. Again, this result is in contrast with the available data in developing mouse. However, the latter data are limited and differences between the species may be temporal rather than spatial. In other studies, iPLA₂-VIA is shown to be expressed in numerous cell lines including macrophages [1], pancreatic cells [31], astrocytes [30] and renal cells [11]. Broad *PLA2G6* expression is consistent with its role in membrane phospholipid maintenance and divergent signaling pathways [3].

Several disorders with predominantly neurodevelopmental defects are caused by genes with a widespread expression pattern during human development [21,37]. In addition, degenerative neurological disorders can be caused by mutations in a widely expressed gene, exemplified by Rett syndrome and pantothenate kinase-associated neurodegeneration [32,40]. Similarly, in several trinucleotide repeat disorders, expanded polyglutamine proteins are ubiquitously expressed but cause selective neurodegeneration in specific brain regions [28].

Our results suggest that *PLA2G6* is positioned to play a role in early neuronal development. Disruption of this process may contribute to the widespread neurological problems observed in INAD. In fact, embryonic and fetal *PLA2G6* expression suggests that INAD pathogenesis could initiate *in-utero*. Several INAD mouse models are available to further investigate the effect of *PLA2G6* mutations on embryonic development [8,20,33,39]. Certainly, *in-utero* *PLA2G6* function should be considered when treatment strategies for INAD are in development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

INAD	infantile neuroaxonal dystrophy
NBIA	neurodegeneration with brain iron accumulation
PCW	post weeks conception
PKAN	pantothenate-kinase associated neurodegeneration
SVZ	subventricular zone
VZ	ventricular zone

Acknowledgments

This work was supported by National Institute of Child Health and Human Development (to S.H.), the Neurodegeneration with Brain Iron Accumulation (NBIA) Disorders Association (to S.H.), the Association Internationale De Dystrophie Neuro Axonale Infantile (AIDNAI) (to S.H.) and the Huebner Family Pediatric Neurobiology of Disease Fellowship (to B.P.). The human embryonic and fetal material was provided by the Joint MRC-Wellcome Trust Human Developmental Biology Resource (<http://www.hdbr.org>) at the IHG, Newcastle-upon-Tyne, UK.

LITERATURE CITED

1. Ackermann EJ, Kempner ES, Dennis EA. Ca(2+)-independent cytosolic phospholipase A2 from macrophage-like P388D1 cells. Isolation and characterization. *J Biol Chem.* 1994; 269:9227–9233. [PubMed: 8132660]
2. Balboa MA, Saez Y, Balsinde J. Calcium-independent phospholipase A2 is required for lysozyme secretion in U937 promonocytes. *J Immunol.* 2003; 170:5276–5280. [PubMed: 12734377]
3. Balsinde J, Balboa MA. Cellular regulation and proposed biological functions of group VIA calcium-independent phospholipase A2 in activated cells. *Cell Signal.* 2005; 17:1052–1062. [PubMed: 15993747]
4. Balsinde J, Perez R, Balboa MA. Calcium-independent phospholipase A2 and apoptosis. *Biochim Biophys Acta.* 2006; 1761:1344–1350. [PubMed: 16962822]
5. Bao S, Miller DJ, Ma Z, Wohltmann M, Eng G, Ramanadham S, Moley K, Turk J. Male mice that do not express group VIA phospholipase A2 produce spermatozoa with impaired motility and have greatly reduced fertility. *J Biol Chem.* 2004; 279:38194–38200. [PubMed: 15252026]
6. Basselin M, Rosa AO, Ramadan E, Cheon Y, Chang L, Chen M, Greenstein D, Wohltmann M, Turk J, Rapoport SI. Imaging decreased brain docosaheanoic acid metabolism and signaling in iPLA2{beta} (VIA)-deficient mice. *J Lipid Res.* 2010
7. Bayer SA. *The Human Brain during the Late First Trimester.* Taylor & Francis. 2006
8. Bouley DM, McIntire JJ, Harris BT, Tolwani RJ, Otto GM, DeKruyff RH, Hayflick SJ. Spontaneous murine neuroaxonal dystrophy: a model of infantile neuroaxonal dystrophy. *J Comp Pathol.* 2006; 134:161–170. [PubMed: 16542671]
9. Bullen, P.; Wilson, D. The Carnegie staging of human embryos: a practical guide. In: Strachan, T.; Lindsay, S.; Wilson, D., editors. *Molecular genetics of early human development.* Oxford: BIOS Scientific Publishers; 1997. p. 27-50.
10. Carnevale KA, Cathcart MK. Calcium-independent phospholipase A(2) is required for human monocyte chemotaxis to monocyte chemoattractant protein 1. *J Immunol.* 2001; 167:3414–3421. [PubMed: 11544333]
11. Cummings BS, Gelasco AK, Kinsey GR, McHowat J, Schnellmann RG. Inactivation of endoplasmic reticulum bound Ca2+-independent phospholipase A2 in renal cells during oxidative stress. *J Am Soc Nephrol.* 2004; 15:1441–1451. [PubMed: 15153555]
12. Dennis EA. Diversity of group types, regulation, and function of phospholipase A2. *J Biol Chem.* 1994; 269:13057–13060. [PubMed: 8175726]
13. Edward DP, Kaufman LM. Anatomy, development, and physiology of the visual system. *Pediatr Clin North Am.* 2003; 50:1–23. [PubMed: 12713101]
14. Gregory A, Westaway SK, Holm IE, Kotzbauer PT, Hogarth P, Sonek S, Coryell JC, Nguyen TM, Nardocci N, Zorzi G, Rodriguez D, Desguerre I, Bertini E, Simonati A, Levinson B, Dias C, Barbot C, Carrilho I, Santos M, Malik I, Gitschier J, Hayflick SJ. Neurodegeneration associated with genetic defects in phospholipase A(2). *Neurology.* 2008; 71:1402–1409. [PubMed: 18799783]
15. Hern WM. Correlation of fetal age and measurements between 10 and 26 weeks of gestation. *Obstet Gynecol.* 1984; 63:26–32. [PubMed: 6691014]
16. Kolko M, Kiilgaard JF, Wang J, Poulsen KA, Andreasen JR, la Cour M, Nissen MH, Heegaard S, Bazan NG, Prause JU. Calcium-independent phospholipase A2 regulates retinal pigment epithelium proliferation and may be important in the pathogenesis of retinal diseases. *Exp Eye Res.* 2009; 89:383–391. [PubMed: 19379734]

17. Kolko M, Wang J, Zhan C, Poulsen KA, Prause JU, Nissen MH, Heegaard S, Bazan NG. Identification of intracellular phospholipases A2 in the human eye: involvement in phagocytosis of photoreceptor outer segments. *Invest Ophthalmol Vis Sci.* 2007; 48:1401–1409. [PubMed: 17325189]
18. Kurian MA, Morgan NV, MacPherson L, Foster K, Peake D, Gupta R, Philip SG, Hendriksz C, Morton JEV, Kingston HM, Rosser EM, Wassmer E, Gissen P, Maher ER. Phenotypic spectrum of neurodegeneration associated with mutations in the PLA2G6 gene (PLAN). *Neurology.* 2008; 70:1623–1629. [PubMed: 18443314]
19. Larsson Forsell PK, Kennedy BP, Claesson HE. The human calcium-independent phospholipase A2 gene multiple enzymes with distinct properties from a single gene. *Eur J Biochem.* 1999; 262:575–585. [PubMed: 10336645]
20. Malik I, Turk J, Mancuso DJ, Montier L, Wohltmann M, Wozniak DF, Schmidt RE, Gross RW, Kotzbauer PT. Disrupted membrane homeostasis and accumulation of ubiquitinated proteins in a mouse model of infantile neuroaxonal dystrophy caused by PLA2G6 mutations. *Am J Pathol.* 2008; 172:406–416. [PubMed: 18202189]
21. Miyake N, Chilton J, Psatha M, Cheng L, Andrews C, Chan WM, Law K, Crosier M, Lindsay S, Cheung M, Allen J, Gutowski NJ, Ellard S, Young E, Iannaccone A, Appukuttan B, Stout JT, Christiansen S, Ciccarelli ML, Baldi A, Campioni M, Zenteno JC, Davenport D, Mariani LE, Sahin M, Guthrie S, Engle EC. Human CHN1 mutations hyperactivate alpha2-chimaerin and cause Duane's retraction syndrome. *Science.* 2008; 321:839–843. [PubMed: 18653847]
22. Moorman AF, Houweling AC, de Boer PA, Christoffels VM. Sensitive nonradioactive detection of mRNA in tissue sections: novel application of the whole-mount in situ hybridization protocol. *J Histochem Cytochem.* 2001; 49:1–8. [PubMed: 11118473]
23. Morgan NV, Westaway SK, Morton JE, Gregory A, Gissen P, Sonek S, Cangul H, Coryell J, Canham N, Nardocci N, Zorzi G, Pasha S, Rodriguez D, Desguerre I, Mubaidin A, Bertini E, Trembath RC, Simonati A, Schanen C, Johnson CA, Levinson B, Woods CG, Wilmot B, Kramer P, Gitschier J, Maher ER, Hayflick SJ. PLA2G6, encoding a phospholipase A2, is mutated in neurodegenerative disorders with high brain iron. *Nat Genet.* 2006; 38:752–754. [PubMed: 16783378]
24. Nardocci N, Zorzi G, Farina L, Binelli S, Scaioli W, Ciano C, Verga L, Angelini L, Savoiardo M, Bugiani O. Infantile neuroaxonal dystrophy: clinical spectrum and diagnostic criteria. *Neurology.* 1999; 52:1472–1478. [PubMed: 10227637]
25. O'Rahilly, R.; Müller, F.; Streeter, GL. Developmental stages in human embryos : including a revision of Streeter's "Horizons" and a survey of the Carnegie collection. Washington, D.C.: Carnegie Institution of Washington; 1987.
26. Ong WY, Yeo JF, Ling SF, Farooqui AA. Distribution of calcium-independent phospholipase A2 (iPLA 2) in monkey brain. *J Neurocytol.* 2005; 34:447–458. [PubMed: 16902765]
27. Ono K, Yasui Y, Rutishauser U, Miller RH. Focal ventricular origin and migration of oligodendrocyte precursors into the chick optic nerve. *Neuron.* 1997; 19:283–292. [PubMed: 9292719]
28. Orr HT, Zoghbi HY. Trinucleotide repeat disorders. *Annu Rev Neurosci.* 2007; 30:575–621. [PubMed: 17417937]
29. Paisan-Ruiz C, Bhatia KP, Li A, Hernandez D, Davis M, Wood NW, Hardy J, Houlden H, Singleton A, Schneider SA. Characterization of PLA2G6 as a locus for dystonia-parkinsonism. *Ann Neurol.* 2009; 65:19–23. [PubMed: 18570303]
30. Peterson B, Knotts T, Cummings BS. Involvement of Ca²⁺-independent phospholipase A2 isoforms in oxidant-induced neural cell death. *Neurotoxicology.* 2007; 28:150–160. [PubMed: 17046062]
31. Ramanadham S, Hsu FF, Zhang S, Jin C, Bohrer A, Song H, Bao S, Ma Z, Turk J. Apoptosis of insulin-secreting cells induced by endoplasmic reticulum stress is amplified by overexpression of group VIA calcium-independent phospholipase A2 (iPLA2 beta) and suppressed by inhibition of iPLA2 beta. *Biochemistry.* 2004; 43:918–930. [PubMed: 14744135]
32. Shahbazian MD, Antalffy B, Armstrong DL, Zoghbi HY. Insight into Rett syndrome: MeCP2 levels display tissue- and cell-specific differences and correlate with neuronal maturation. *Hum Mol Genet.* 2002; 11:115–124. [PubMed: 11809720]

33. Shinzawa K, Sumi H, Ikawa M, Matsuoka Y, Okabe M, Sakoda S, Tsujimoto Y. Neuroaxonal dystrophy caused by group VIA phospholipase A2 deficiency in mice: a model of human neurodegenerative disease. *J Neurosci.* 2008; 28:2212–2220. [PubMed: 18305254]
34. Shirai Y, Ito M. Specific differential expression of phospholipase A2 subtypes in rat cerebellum. *J Neurocytol.* 2004; 33:297–307. [PubMed: 15475685]
35. Small RK, Riddle P, Noble M. Evidence for migration of oligodendrocyte--type-2 astrocyte progenitor cells into the developing rat optic nerve. *Nature.* 1987; 328:155–157. [PubMed: 3600791]
36. Sun GY, Shelat PB, Jensen MB, He Y, Sun AY, Simonyi A. Phospholipases A2 and Inflammatory Responses in the Central Nervous System. *Neuromolecular Med.* 2009
37. Tischfield MA, Baris HN, Wu C, Rudolph G, Van Maldergem L, He W, Chan WM, Andrews C, Demer JL, Robertson RL, Mackey DA, Ruddle JB, Bird TD, Gottlob I, Pieh C, Traboulsi EI, Pomeroy SL, Hunter DG, Soul JS, Newlin A, Sabol LJ, Doherty EJ, de Uzcategui CE, de Uzcategui N, Collins ML, Sener EC, Wabbels B, Hellebrand H, Meitinger T, de Berardinis T, Magli A, Schiavi C, Pastore-Trossello M, Koc F, Wong AM, Levin AV, Geraghty MT, Descartes M, Flaherty M, Jamieson RV, Moller HU, Meuthen I, Callen DF, Kerwin J, Lindsay S, Meindl A, Gupta ML Jr, Pellman D, Engle EC. Human TUBB3 mutations perturb microtubule dynamics, kinesin interactions, and axon guidance. *Cell.* 140:74–87. [PubMed: 20074521]
38. Visel A, Thaller C, Eichele G. GenePaint.org: an atlas of gene expression patterns in the mouse embryo. *Nucleic Acids Res.* 2004; 32:D552–D556. [PubMed: 14681479]
39. Wada H, Yasuda T, Miura I, Watabe K, Sawa C, Kamijuku H, Kojo S, Taniguchi M, Nishino I, Wakana S, Yoshida H, Seino K. Establishment of an improved mouse model for infantile neuroaxonal dystrophy that shows early disease onset and bears a point mutation in *Pla2g6*. *Am J Pathol.* 2009; 175:2257–2263. [PubMed: 19893029]
40. Zhou B, Westaway SK, Levinson B, Johnson MA, Gitschier J, Hayflick SJ. A novel pantothenate kinase gene (*PANK2*) is defective in Hallervorden- Spatz syndrome. *Nat Genet.* 2001; 28:345–349. [PubMed: 11479594]

Figure 1.

PLA2G6 expression in human brain at CS19, CS23 and 9 PCW. Panels A–L show images of *in situ* hybridization with antisense probes for *PLA2G6* to sections at CS19 (A, J), CS23 (B, D–I) and 9PCW (C, K, L). Signal is detected as a purple stain. **A)–C)** *PLA2G6* staining in developing neocortex. At CS19 (A), expression can be seen close to the lateral ventricle (VZ), by CS23 (B) expression is in the VZ and SVZ and by 9PCW (C) expression is also seen in the CP. **D)** Low magnification image showing developing neocortex, ganglionic eminences and thalamus. The boxed areas are shown at higher magnification in panels **E** and **F**. **G)** Low magnification image showing developing midbrain, isthmus, cerebellum and pons. The boxed areas are shown at higher magnification in panels **H** and **I**. **J)** *PLA2G6* expression is seen in the alar plate of the developing midbrain. **K)** *PLA2G6* expression in the hindbrain is seen in the developing cerebellum, the VZ of medulla and neurons in developing nuclei (IO). Boxed area is seen at higher magnification in panel **L**. No signal was detected using sense control probes (supplemental figure 1). C- cerebellum, CP- cortical plate; GE- ganglionic eminences, IO- inferior olive, I- isthmus, LV- lateral ventricle, M- medulla, MB- midbrain, NC- neocortex, P= pons, SVZ- subventricular zone, T- thalamus, VZ- ventricular zone. Scale bars are: 100µm in A,C,J,L; 200µm in B,E,F,H,I; 1000µm in D,G,K.

Figure 2.

PLA2G6 expression in human eye and spinal cord at CS19 (A,E) CS23 (B, F) and 9PCW (C,D). Each panel has 2 images, the top one showing *in situ* hybridization using antisense (AS) probes and the lower one using sense (S) probes for *PLA2G6*. No signal was detected using sense control probes **A)** *PLA2G6* staining in retina and lens at CS19. **B)** *PLA2G6* staining in lens, retina and optic nerve at CS23. **C–D)** *PLA2G6* staining in retina at PCW9. **E)** *PLA2G6* staining in dorsal root ganglia at CS19. **F)** *PLA2G6* staining in dorsal root ganglia at 9PCW A- alar plate, AS- antisense, DRG- dorsal root ganglia, L- lens, ON- optic nerve, R-retina, S-sense, Scale bars are: 100 μ m in A,E; 200 μ m in B,C,D,F.

Figure 3.

Expression of *PLA2G6* in non-neuronal tissues. Each panel has 2 images, the top one showing *in situ* hybridization using AS probes and the lower one using S probes for *PLA2G6*. No signal was detected using sense control probes. Panels A, C, E and F are CS23 sections while Panels B, D and G sections at 9PCW. **A)** Lung **B)** Metanephros **C)** Gonad and mesonephros **D)** Tongue **E)** Nasal area **F)** Upper limb **G)** Intestine **H)** Eye muscle. AS- antisense, NE- nasal epithelium, OM=- ocular muscles, S- sense. Scale bars are: 100 μ m in B,C,D,G; 200 μ m in A,E,F,H.