STUDY PROTOCOL

Background

Neurodegeneration with Brain Iron Accumulation (NBIA) comprises a heterogeneous group of rare disorders characterised by the deposition of brain iron (ref). Phospholipase A2-associated Neurodegeneration (PLAN) is one of the major NBIA subtypes, accounting for 20%of patients with NBIA[1]

Clinical phenotypes[1, 2]

PLAN comprises a continuum of three phenotypes with overlapping clinical and radiologic features:

- Classic infantile neuroaxonal dystrophy (INAD)
- Atypical neuroaxonal dystrophy (atypical NAD)
- PLA2G6-related dystonia-parkinsonism

INAD usually manifests between six months and three years of age, with developmental regression and hypotonia. Over time, children develop psychomotor delay and progressive spastic tetraparesis. Eye features (strabismus, nystagmus, and optic atrophy) are commonly reported. Many affected children either never learn to walk or lose independent ambulation over time. Disease progression is usually rapid and death typically occurs during the first decade of life.

Atypical NAD shows more phenotypic variability than INAD. In general, disease onset is in early childhood but may be as late as the end of the second decade. Presenting features include gait instability, ataxia, speech delay and autistic traits. The clinical course is usually more slowly progressive than that observed in classical INAD, with neurologic deterioration in late childhood and adolescence.

PLA2G6-related dystonia-parkinsonism presents with subacute onset of dystonia-parkinsonism in late adolescence/early adulthood. Patients often show additional eye movement abnormalities, pyramidal tract signs, and marked cognitive decline over time.

Radiological features

For INAD, a number of typical MRI features are described (**Figure 1**), including

- Cerebellar cortical atrophy and gliosis
- T2-weighted hypointensity within the globus pallidus and substantia nigra consistent with iron deposition
- Reduced volume in the optic chiasm and optic nerves

White matter changes and posterior corpus callosal abnormalities are also often reported.

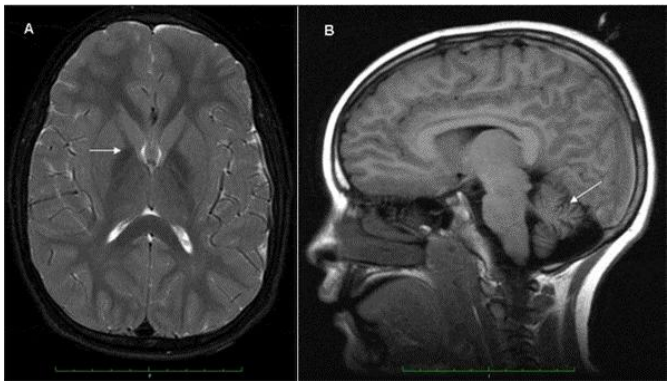

Figure 1. Magnetic Resonance Imaging in PLAN.
A: axial T2-weighted image depicting decreased signal in the globus pallidi bilaterally consistent with iron deposition. B: sagittal T1-weighted image indicating cerebellar atrophy.

Investigations

In classical INAD, the following features can be seen on diagnostic investigations:

- EEG: widespread high amplitude fast activity (at 16 to 22 Hz) in the majority of patients.
- EMG/ Nerve Conduction Studies: electromyographic signs of denervation are very frequent. Additionally, distal axonal-type sensorimotor neuropathy is evident in 43% of patients.
- Visual Evoked Potentials: absent or delayed in the majority of children examined.
- Sural nerve and skin biopsy studies: features of axonal swelling and spheroid body formation often shown.
- LDH (lactate dehydrogenase) and AST (aspartate aminotransferase): also found to be elevated/ abnormal in a number of patients but no substantial data regarding these biomarkers exist in the literature to date.

Molecular Genetics

PLAN is due to autosomal recessive mutations in *PLA2G6.* Disease mechanisms remain yet to be fully elucidated. The protein has a role in controlling membrane phospholipid turnover and mass; PLA2G6 has also been shown to be expressed in the mitochondria and be protective to mitochondrial health. It is postulated that gene mutations lead to perturbation of membrane lipid homeostasis and mitochondrial function with resultant axonal pathology and neurodegeneration.

Rationale for PLAN Natural History Study

PLAN is a progressive neurodegenerative disorder, and there are currently no available drugs that can improve or cure this condition. Indeed current treatment strategies focus only on a symptomatic approach.

At UCL, we are developing a novel therapeutic approach for PLAN, by exploring a proof-of-concept gene therapy strategy in the INAD knock-in mouse model. Preliminary results are

promising, and we have been able to demonstrate rescue of the mouse model (which recapitulates a number of the motor features evident in human patients) using an AAV9 vector delivery system. Further work is now underway to bring this work towards clinical translation.

Given these advances in developing novel therapeutic strategies for PLAN, there is now urgent clinical need to gather important clinical data regarding the natural disease course in order to be 'trial-ready' for future gene therapy and/or other novel therapeutic trials

Objectives of Proposed BPNSU Surveillance Study

As a rare disease, PLAN is poorly understood, and there is currently limited published data regarding disease course and long-term outcome in patients with this disease. A natural history study is therefore important in order to:-

- Accurately track disease course over time and establish long-term survival data to better understand the disease trajectory of PLAN. Such data will be imperative for assessing the efficacy of novel treatments in future clinical trials
- Identify demographic, genetic, environmental and other variables that may correlate with disease outcomes
- Identify best practices in patient care (consensus expert opinion) to develop diagnostic/management guidelines in the future
- Identify research priorities
- Identify potential available disease biomarkers that may be associated with disease progression (e.g. LDH, AST)

Study Design and Methodology

(1) Recruitment of Local GOSH Patients
Patients with PLAN from Great Ormond Street Hospital (GOSH) will be recruited into the study by undertaking a local audit to identify all GOSH cases. Anonymised data will be collected on site (by reviewing medical case notes and neuroimaging). Data will be recorded on a standardised anonymised proforma (see attached).

(2) Recruitment of Cases from the UK and Ireland
The project will be advertised through the BPNSU main website and also through the British Paediatric Neurology Association website. Regular newsletters and emails will be sent to BPNA members. The anonymised proforma for data collection will be uploaded to the BPNSU webpage to enable participating clinicians from UK centres to complete and return the required data. Dr Apostolos Papandreou and Dr Manju Kurian will co-ordinate and oversee data collection from clinicians in different UK centres.

(3) Inclusion and Exclusion Criteria

Case definition: Patients with a clinical phenotype consistent with phospholipase A2-associated neurodegeneration (PLAN), harbouring disease-causing mutations in the *PLA2G6* gene

Age range for cases:

- Lower limit: 0 years
- No upper age limit

Inclusion criteria:

- Patients with a confirmed diagnosis of PLAN residing in the UK/Ireland

- Clinical phenotypes will include

1. Classic infantile neuroaxonal dystrophy (INAD)
2. Atypical neuroaxonal dystrophy (atypical NAD)
3. *PLA2G6*-related dystonia-parkinsonism

- Two pathogenic mutations in *PLA2G6* gene

Exclusion criteria:

- Patients where the diagnosis of PLAN has not been fully and comprehensively established by the identification of two disease-causing mutations in *PLA2G6*
- Other NBIA subtypes

(4) Data Collation and Analysis

Data collected with the standardised anonymised proforma will be entered anonymously into our PLAN database at GOS-Institute of Child Health, which is password-protected and fully anonymized. Each recruited case will be given an anonymised identifier number. Once anonymised data has been collected, it will be collated and analysed with the aim to establish the natural disease course of PLAN, from the pre-symptomatic phase through subsequent clinical stages.

Specifically we aim to establish:

- Age of disease presentation
- Age at which specific regression and neurodevelopmental skill loss occurs
- Age at loss of independent ambulation
- Age of onset of strabismus and evolution of other eye features
- Age of onset of bulbar dysfunction
- Age of onset of behavioural difficulties and/ or psychiatric features
- Age of onset of dystonia and other movement disorders
- Age of onset of epilepsy
- Presence of axonal neuropathy on nerve conduction studies
- Evidence of fast activity on EEG
- Abnormal VER
- Results of AST/LDH testing
- Long term survival data, and age of death (Kaplan-Meier survival curves)

(5) Funding

In order to register the project with the BPNSU for 24 months, £400 will be required. This funding has already been secured through a grant from the NBIA Disorders Association. No additional funding will be required. Dr Kurian and Dr Papandreou will be responsible for overseeing and undertaking the study; both are currently funded by external research fellowships.

REFERENCES

1. Illingworth MA, Meyer E, Chong WK, et al. PLA2G6-associated neurodegeneration (PLAN): further expansion of the clinical, radiological and mutation spectrum associated with infantile and atypical childhood-onset disease. Mol Genet Metab 2014;112:183-189.
2. Kurian MA, Morgan NV, MacPherson L, et al. Phenotypic spectrum of neurodegeneration associated with mutations in the PLA2G6 gene (PLAN). Neurology 2008;70:1623-1629.