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Brief Communication

Multiplex ligation-dependent probe amplification (MLPA) analysis is an effective tool for the detection of novel intragenic *PLA2G6* mutations: Implications for molecular diagnosis

Danielle Crompton [a], Pauline K. Rehal [a], Lesley MacPherson [b], Katharine Foster [b], Peter Lunt [c], Imelda Hughes [d], Angela F. Brady [e], Michael G. Pike [f], Susanna De Gressi [g], Neil V. Morgan [h], Carol Hardy [a], Matthew Smith [a], Fiona MacDonald [a], Eamonn R. Maher [a,h], Manju A. Kurian [h,i,*]

[a] *West Midlands Regional Genetic Service, Birmingham Women's Hospital, Metchley Park Lane, Edgbaston, Birmingham B15 2TG, UK*
[b] *Department of Radiology, Birmingham Children's Hospital, Steelhouse Lane, Birmingham B4 6NH, UK*
[c] *Department of Clinical Genetics, St. Michael's Hospital, Southwell Street, Bristol BS2 8EG, UK*
[d] *Department of Paediatric Neurology, Royal Manchester Children's Hospital, Oxford Road, Manchester M13 9WL, UK*
[e] *North West Thames Regional Genetics Service, Kennedy-Galton Centre, Northwick Park Hospital, Watford Road, Harrow HA1 3UJ, UK*
[f] *Department of Paediatric Neurology, John Radcliffe Hospital, Oxford OX3 9DU, UK*
[g] *Department of Paediatrics, Cheltenham General Hospital, Gloucestershire GL53 7AN, UK*
[h] *Department of Medical and Molecular Genetics, University of Birmingham School of Medicine, Institute of Biomedical Research, Birmingham B15 2TT, UK*
[i] *Department of Paediatric Neurology, Birmingham Children's Hospital, Steelhouse Lane, Birmingham B4 6NH, UK*

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ABSTRACT

Phospholipase associated neurodegeneration (PLAN) comprises a heterogeneous group of autosomal recessive neurological disorders caused by mutations in the *PLA2G6* gene. Direct gene sequencing detects ~85% mutations in infantile neuroaxonal dystrophy. We report the novel use of multiplex ligation-dependent probe amplification (MLPA) analysis to detect novel *PLA2G6* duplications and deletions. The identification of such copy number variants (CNVs) expands the PLAN mutation spectrum and may account for up to 12.5% of *PLA2G6* mutations. MLPA should thus be employed to detect CNVs of *PLA2G6* in patients who show clinical features of PLAN but in whom both disease-causing mutations cannot be identified on routine sequencing.

Introduction

Autosomal recessive neurodegeneration associated with genetic defects in the *PLA2G6* gene [1] may present with a number of phenotypes [2] including presentation in infancy [3,4] (infantile neuroaxonal dystrophy, INAD, MIM256600), childhood (atypical neuroaxonal dystrophy, neurodegeneration with brain iron accumulation, NBIA MIM610217, Karak syndrome MIM608395) [4,5] and also in adulthood [6,7] (early-onset dystonia-parkinsonism MIM612953). Mutation detection rate is particularly high (80–90%) in children with classical clinical and radiological features of infantile PLAN [2,8].[1]

Classical INAD (infantile onset PLAN) accounts for the majority of cases, and is characterised by infantile onset truncal hypotonia and progressive psychomotor regression [3,4]. Over time, children develop bulbar dysfunction, pyramidal tract signs, optic atrophy, cerebellar features and extrapyramidal features [3,4,9–12]. MRI features can aid diagnosis [13–15]. The majority of patients have features of cerebellar atrophy [4]. Cerebellar gliosis is seen in the majority [4] but not all patients have this feature [16]. Some also have evidence of brain iron accumulation [3,4]. Generalised seizures are often reported [17–19]. Dysmorphia is rarely described [20]. Death usually occurs around the first decade [3,4].

* Corresponding author. Address: 2nd Floor Norton Court, Birmingham Women's Hospital, Metchley Park Road, Edgbaston, Birmingham B15 2TG, UK.
E-mail addresses: manju_dave@hotmail.com, m.kurian@bham.ac.uk (M.A. Kurian).

[1] *Abbreviations used:* PLAN, phospholipase associated neurodegeneration; MLPA, multiplex ligation-dependent probe amplification; ARMD, Alu recombination-mediated deletion.

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Not all patients with typical clinical features of infantile PLAN have mutations in the *PLA2G6* gene [4]. Possible explanations for this include genetic heterogeneity [1], *PLA2G6* defects within intronic sequence or regulatory regions and CNVs that are undetected by standard diagnostic mutational screening strategies [21]. In recent years, MLPA has emerged as a high resolution technique to determine relative DNA sequence dosage [22,23].

We describe four children with infantile PLAN referred for diagnostic *PLA2G6* screening in which both disease-causing mutations were not identified on direct gene sequencing. Further investigation with MLPA analysis detected a novel heterozygous duplication in patient 1 and a novel homozygous deletion in patients 2–4.

Subjects and methods

Patients

The patients described were referred to the West Midlands Regional Genetic Service for *PLA2G6* analysis by their local paediatric neurologist/geneticist. The medical case notes were analysed to delineate the clinical features on history and examination. MRI brain scans for patient 1 were reviewed independently by 2 paediatric neuroradiologists (with consensus agreement on disparities).

Molecular genetic investigation

Techniques for DNA/RNA extraction, *PLA2G6* sequencing, MLPA analysis and molecular characterisation of the CNVs are outlined in Supplementary data 1.

Results

Clinical cases

Patient 1

Patient 1 was the first child of non-consanguineous healthy Caucasian parents. Early neurodevelopmental milestones were achieved. At 15 months, he developed gait instability and an alternating strabismus. Psychomotor regression ensued and by 19–20 months of age, there was loss of ambulation. Between 2 and 3 years he developed 4-limb spasticity. Speech regression was also evident. He developed severe bulbar dysfunction with excessive drooling and feeding difficulties requiring PEG feeding. Dystonia of all 4-limbs was also evident by age 5 years. He did not have any seizures.

On clinical examination (age 5 years) he was not dysmorphic or microcephalic, but had evidence of a right-sided manifest squint and bilateral horizontal nystagmus. There was excessive drooling and tongue fasciculation. On neurological assessment he was found to have marked axial hypotonia. There was a postural kyphotic curvature of the spine. Limb examination revealed symmetrical 4-limb hypertonicity and hyperreflexia but no contractures. Plantar reflexes were upgoing bilaterally.

MRI brain examination was undertaken at age 2 and 2.9 years (Fig. 1). Initial MRI scan showed evidence of a hypoplastic cerebellum and vermis (Fig. 1A and B). There was no evidence of optic nerve hypoplasia or white matter abnormalities. On repeat MR neuroimaging at age 2.9 years, there was progressive cerebellar atrophy (Fig. 1C and D). Although minimal basal ganglia abnormalities were noted on initial imaging (Fig. 1E), mild iron accumulation within the basal ganglia was evident on repeat imaging (Fig. 1F). Electromyographic (EMG) signs of chronic denervation were evident in the upper limbs, lower limbs and bulbar musculature. Nerve conduction studies were normal. On EEG, widespread high amplitude fast activity at 16–22 Hz was seen. Visual evoked potentials (VEP) and electroretinogram (ERG) was normal at age 2 years. Ophthalmological review detected bilateral temporal disc pallor. Histological examination of nerve tissue from a rectal biopsy (age 2 years) was normal.

Patient 2

Patient 2, a 4 year old girl was born to healthy consanguineous (2nd cousin) Irish parents. All early developmental milestones were appropriately achieved. Symptom onset at 9–10 months of age commenced with psychomotor regression. She stopped vocalising, developed complete loss of ambulation and could only sit for brief periods with extensive support. There was no evidence of seizures.

On clinical examination, at age 21 months, she was not dysmorphic or microcephalic. There was evidence of marked axial hypotonia and upper and lower limb spasticity (but no contractures or spinal deformity). She had evidence of nystagmus on clinical examination. Ophthalmological examination did not detect any abnormalities.

MRI brain (age 18 months) revealed cerebellar atrophy and a marginally narrow pons but no evidence of basal ganglia abnormalities. Electromyogram abnormalities were similar to patient 1. She did not have any other electrophysiological investigations (VER, ERG, EEG) or a sural/skin biopsy as repeated non-attendance at clinic appointments precluded further neurological investigation.

Patients 3 and 4

Patients 3 and 4, dizygotic male twins were born to healthy consanguineous (1st cousin) parents, of Irish origin. There was no family history of neurological disorders and the family were not known to be related to patient 2's kindred. The twins were born at 33 weeks gestation, but despite their prematurity, the postnatal course was uneventful. Early developmental milestones were appropriately achieved, but concerns were raised at 11 months of age as both children were yet to sit independently. At this stage both children were noted to have strabismus. Psychomotor regression ensued in both children with a gradual but progressive loss of cognitive and motor skills. A rapid decline in motor function was seen in patient 3 subsequent to a febrile viral illness at age 22 months. Following this illness, he developed infantile spasms associated with a hypsarrhythmic EEG pattern. At age 26 months, patient 4 also developed seizures (generalised tonic clonic episodes) with an EEG pattern of high voltage slow background with sharp and slow wave discharges seen independently in each temporal region. Repeat EEG (5 months later) showed mild excess of moderate voltage irregular slow activity with runs of high amplitude irregular delta activity with ill defined sharp components independently over both temporal regions. Both children had ophthalmological assessments showing optic atrophy. Over time, both children developed profound axial hypotonia, pyramidal tract features and bulbar dysfunction.

MRI brain (at 24 months of age in both children) revealed moderate cerebellar atrophy, but no cerebellar gliosis or brain iron accumulation. Electrophysiological investigation revealed absent sensory nerve action potentials associated with a myopathic pattern on EMG. No clear binocular pattern of vision was evident on VEP. A sural/skin biopsy was not undertaken in either patient.

Molecular genetic investigation

Direct sequencing of PLA2G6

Patient 1: A heterozygous mutation (c.1674delG; p.Leu560TrpfsX5) was detected in exon 12 (Supplementary Fig. 1). A 2nd mutation was not detected.

Patient 2–4: Molecular analysis did not detect any mutations in exons 1–4 and 7–17 of the *PLA2G6* gene. Repeated failed attempts