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Molecular Diagnosis of Infantile Neuro axonal Dystrophy by Next Generation Sequencing

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Abstract

Infantile Neuro axonal Dystrophy (INAD), is a rare inherited neurological disorder which affects nerve axons causing progressive loss of mental skills, muscular control and vision. The authors present a case of 5.8-y-old girl with INAD who was diagnosed after Next Generation Sequencing

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Conflict of Interest None.

(NGS). She was born to a non-consanguineous couple and presented with hypotonia, developmental delay followed by neuroregression and nystagmus after 2 years of age. On examination, bilateral horizontal nystagmus and normal head circumference were noted. Brain MRI showed cerebellar atrophy and altered signal intensities in bilateral globus pallidi and thalami. Magnetic resonance spectroscopy (MRS) showed elevation of lactate. Metabolic testing with Tandem Mass Spectrometry (TMS) and Gas Chromatography Mass Spectrometry (GC-MS) were normal. Mitochondrial disorder was suspected in view of clinical presentation, increased lactate and neuro-imaging suggestive of Leigh syndrome. Mitochondrial Leigh mutations and *SURF1* gene sequencing yielded normal results. Lack of a clear diagnosis led to performance of NGS using panel of about 514 genes. A homozygous novel mutation at position c.2277-1G>C in *PLA2G6* gene presumed to give rise to altered splicing, was detected, thus confirming the diagnosis of INAD. This report provides evidence of the usefulness of NGS technology as a quick and accurate diagnostic tool for an otherwise complicated genetic disease. To the authors knowledge, this is the first case report with mutations in *PLA2G6* gene from India.

Keywords

Infantile neuro axonal dystrophy; *PLA2G6* gene; India; Next Generation Sequencing; NGS; Neuronal brain iron accumulation

Introduction

Infantile neuroaxonal dystrophy (INAD) (MIM 256600) is a rare autosomal recessive neurodegenerative disease characterized by pathologic axonal swelling and spheroid bodies in the central nervous system (CNS) [1]. Onset is within the first 2 years of life, and the disease is characterized by progressive loss of cognitive and motor skills, bulbar dysfunction, strabismus and axial hypotonia with four limbs spasticity [2]. Mutations in *PLA2G6* gene have been shown to be causative for INAD [3]. The authors report a case with psychomotor regression and hypotonia with a homozygous splice acceptor mutation, c. 2277-1G>C in *PLA2G6* gene. This is a novel mutation predicted to be pathogenic by giving rise to altered splicing. The authors also report the utility of next generation sequencing (NGS) technology in providing a molecular diagnosis in cases where a specific clinical diagnosis is difficult to make.

Case Report

The proband was born to non-consanguineous couple, at term after normal vaginal delivery with birth weight of 2.7 kg. She was the first child, and had started sitting, crawling and standing with support at appropriate ages. Thereafter, a delay in development was noted at about 15 months of age at which time she had started taking a few steps with support, and had developed babbling speech. She had reasonable understanding, smiled and recognized parents at 2 years of age. The development remained static in second year of life. Bilateral nystagmus was noted at 2 years of age. After 2 years, there was a significant regression in motor and cognitive skills so much so that she lost control of neck and spine at 3 years of age. On last examination at 5.8 years of age, she was barely able to sit with support, had no

speech or interaction including recognition of parents. There was no history of seizures, visual or hearing deficit. There was history of few jerky movements around the time of sleep, but no frank seizures.

On examination at first visit at 3.4 years of age, there were no dysmorphic features. Frog-like posturing with peripheral contractures at ankles was noted. There was bilateral horizontal nystagmus. Her weight, length and head circumference were 11 kg (25th -50th centile), 95 cm (50th centile) and 51 cm (50th -95th centile), respectively. There was no hepato-splenomegaly or any neurocutaneous stigmata. Her body tone was variable with intermittent tightening. Deep tendon reflexes were normal in upper limbs and sluggish in lower limbs with extensor planter at ankle joint.

Blood investigations including serum TSH, free T4, free T3 were 2.95 mIU/L, 13.36 pmol/L and 4.95 pmol/L respectively. Arterial lactate and ammonia were 24 mg/dl (ref. 4.5–20 mg/dl) and 40 µmol/L (ref. 9–35 µmol/L) respectively. Renal function test, liver function test, Visual evoked potentials (VEP), Brainstem evoked response audiometry (BERA) and Nerve conduction velocity (NCV) were normal. Disk pallor was noted on fundus examination. Enzyme assays for Metachromatic leukodystrophy (Arylsulphatase A) and Krabbe disease (beta- Galactocerebrosidase) were normal. MRI of the brain showed cerebellar atrophy and altered signal intensities in bilateral globus pallidi and thalami (Fig. 1). MRS showed a lactate doublet. Metabolic investigations including Tandem Mass Spectrometry (TMS) and urine Gas Chromatography Mass Spectrometry (GC-MS) showed no specific etiology except mildly increased lactate on GC-MS. In view of clinical presentation, increased lactate in urine, and peculiar MRI / MRS findings, a mitochondrial disorder was suspected. Mitochondrial Leigh mutations and *SURF1* gene sequencing yielded normal results. *PANK2* gene was partially studied (common Agarwal mutation) yielding no mutation. Next generation sequencing was then performed in view of no definitive diagnosis.

DNA was prepared for sequencing, utilizing the Illumina TruSeq library preparation kit followed by Illumina TruSeq custom enrichment for 514 genes (Illumina, San Diego, CA). Enrichment efficiency was verified using quantitative real-time PCR. Samples were sequenced on an Illumina HiSeq 2000 instrument with TruSeq v3 reagents, yielding paired end 100 base pair reads. Alignment and variant calling was performed as previously reported [4]. Briefly, gapped alignment to reference sequences (Hg 19 and GRCH37 [NC_012920.1] was performed with GSNAP and GATK). Nucleotide variants were genotyped and annotated utilizing RUNES. Variant analysis for all samples was completed utilizing Viking.

The patient was found to have a novel, homozygous splice acceptor site mutation at position c.2277-1G>C in *PLA2G6* gene, confirming the diagnosis of INAD.

Discussion

INAD is one of the *PLA2G6*-associated neurodegeneration (PLAN) group of disorders amongst the category of ‘neuro-degeneration associated with brain iron accumulation’

(NBIA) in the basal ganglia [2]. The major clinical features for INAD are psychomotor regression, cerebellar atrophy, and optic atrophy with characteristic pattern of early truncal hypotonia followed by the development of spastic tetraparesis [5]. The neuroradiologic hallmark of INAD is cerebellar atrophy and signal hyperintensity in the cerebellar cortex and altered signal intensity in the globus pallidus and substantia nigra on MR T2-weighted images [6]. Pathological features are axonal swellings and spheroids throughout the central nervous system and sometimes spheroids in peripheral tissues [7].

Phospholipase A2 dysfunction is a causative factor of neurodegeneration and disease associated with brain iron dyshomeostasis [3]. *PLA2G6* encodes iPLA2-VI, a calcium-independent phospholipase, with proposed roles in phospholipid remodeling, arachidonic acid release, leukotriene and prostaglandin synthesis and apoptosis [8]. Defects in iPLA2-VIA can lead to a relative abundance of membrane phosphatidyl choline and secondary structural abnormalities, which may underlie the axonal pathology observed in INAD and NBIA. *PLA2G6* mutations predicted to lead to absent protein, are associated with the early onset and rapid progression of INAD, whereas compound heterozygous missense mutations are associated with the less severe phenotype of NBIA [9].

The index case had all the classical features of INAD and detection of mutation in *PLA2G6* gene provided the genetic confirmation of the diagnosis by NGS. The family benefited from the molecular diagnosis as an accurate prenatal diagnosis can now be offered to the couple to rule out the 25 % risk of recurrence. An extended carrier screening can also be offered in the family for reproductive decisions.

The mutation detected is a novel and not-previously reported one, although splice site mutations have been reported in Human Genome Variation Database. To the authors' knowledge, this is the first case report of INAD, with mutations in *PLA2G6* gene, from India. The diagnosis of the present case was greatly helped by use of NGS technology where multiple genes are tested simultaneously, thus providing both cost effective and time saving strategy for molecular diagnosis in difficult cases.

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Fig. 1. MRI of the brain showing altered signal intensities in basal ganglia (hypointensity in bilateral globus pallidus and thalami and hyperintensity in caudate and anterior putamen on T2-weighted image, **a & b** and cerebellar hypoplasia **c & d**)