Fatty Acid Hydroxylase- Associated Neurodegeneration - A Rare Case of Neurodegeneration with Brain Iron Accumulation (NBIA)

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Abstract

Fatty acid hydroxylase associated neurodegeneration is a rare disorder which belongs to the group of disorders of neurodegeneration with brain iron accumulation (NBIA). We present a case of a 9-year-old girl who presented with gradually progressive stiffness of limbs with speech delay and neuroimaging findings of T2 hypointensities in the globus pallidus and substantia nigra suggestive of brain iron accumulation. Targeted exome sequencing by next generation sequencing (NGS) revealed a novel homozygous splice site likely pathogenic variant in intron 6 of the *FA2H* at position c.1039+2T>G, confirming the diagnosis of Fatty acid hydroxylase-associated neurodegeneration (FAHN), a subtype of NBIA. FAHN has never been reported from the Indian subcontinent before. This report further emphasizes the use of good clinical evaluation and NGS in diagnosing rare disorders which otherwise are difficult to recognise.

Introduction

Iron is vital to life but it may generate neurotoxic reactive oxygen species if inappropriately handled. Neurodegeneration with brain iron accumulation (NBIA) is a group of inherited neurologic disorders in which iron accumulates in the basal ganglia (most often in the globus pallidus and/or substantia nigra) resulting in variable degree of progressive dystonia, spasticity, parkinsonism, neuropsychiatric abnormalities, with or without optic atrophy or retinal degeneration [Gregory et al., 2018]. This disorder has 10 subtypes based on involvement of different genes. It is a slowly progressive disorder with marked genetic and clinical heterogeneity. We present a patient with progressive motor regression with spasticity and mild optic atrophy due to novel biallelic variant in the *FA2H* gene (Fatty acid hydroxylase associated Neurodegeneration, FAHN), affecting GT donor splice site in intron 6. The role of thorough clinical examination, high level of suspicion and the use of next generation sequencing (NGS) is emphasized in this report.

Case report

The proband was a 9-year-old girl, the second child of non-consanguineous parents from Bangladesh, born by Caesarean section at term gestation, with a birth weight of 2.8 kg. She required no special care at birth or in the perinatal period. She achieved developmental milestones appropriately till 2 years of age, after which the parents noted some difficulties in mobility. Initially she used to run on toes but then her condition worsened and the difficulty became evident in walking as well. She was able to walk independently till 4 years of age, which at the time of examination regressed to walking a few steps with great difficulty, using support.

There was speech delay, although she started speaking monosyllables at 2 years, no further progress in speech was noted and at 9 years, she had unclear speech with only few words in her vocabulary. Hearing evaluation showed normal result. There was no history of any seizure or acute illness associated with lethargy or coma at any time. No history of visual impairment or abnormal movement of eyes (nystagmus) was given. Her intellect appeared normal, although it was not formally assessed.

On examination, she was alert and cooperative and her anthropometric measurements were corresponding to 25th centile for the age (as per WHO standards). On general examination, no

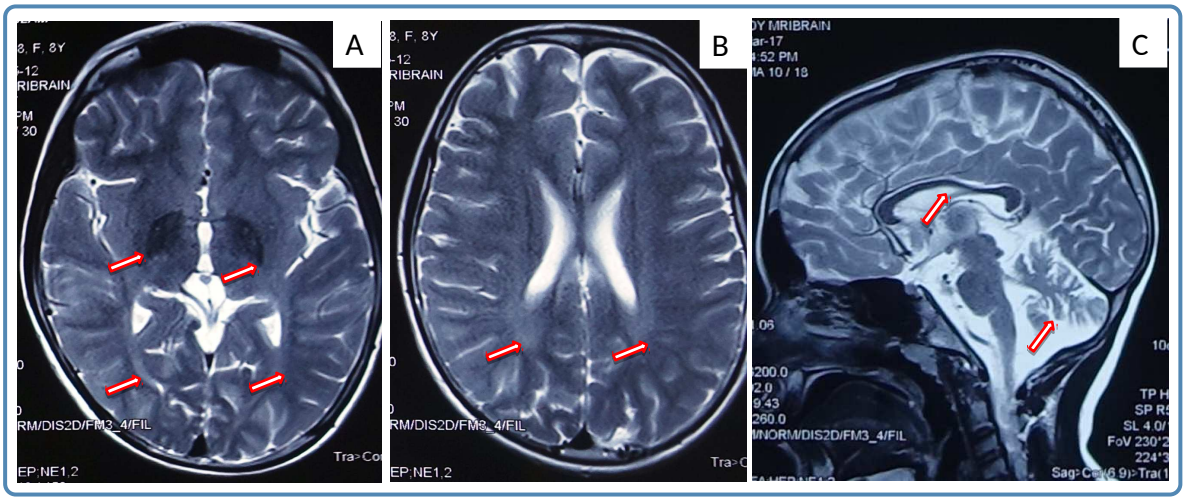

Figure 1 MRI of the brain (T2 images) showing hypointensity in bilateral globus pallidus (Fig 1A), white matter hyperintensities (Fig 1A & 1B), thin corpus callosum and cerebellar hypoplasia (Fig 1C). Figures A & B axial view, figure C) sagittal view.

dysmorphic features were present. The respiratory and cardiovascular system and abdominal examination was normal. On central nervous system (CNS) examination, generalised spasticity was noted involving distal joints of lower limbs more than upper limbs. Power was normal, and deep tendon reflexes were brisk in both the upper and lower limbs. On eye evaluation, mild optic atrophy was noted. There was no nystagmus or KF ring.

Parents had sought medical consultation 6-months prior at which time she had been evaluated. Plasma lactate and ammonia were 23 mg/dl (ref. 4.5–20 mg/dl) and 54 µmol/L (ref 9–35 µmol/L) respectively. Thyroid profile, renal function tests and liver function tests were normal. Metabolic investigations including Tandem Mass Spectrometry (TMS) and urine Gas Chromatography Mass Spectrometry (GC-MS) showed no specific etiology. Eye evaluation showed mild disc pallor suggestive of optic atrophy. Visual evoked potential test revealed mild delay in amplitude and latency in both the eyes further confirming the diagnosis of optic atrophy. Brain MRI showed mild prominence of cerebellar foliae, ponto-cerebellar hypoplasia and white matter hyperintensities on bilateral parieto-occipital regions. Globus pallidus and substantia nigra hypointensities were appreciated in T2 weighted images (Figure 1).

Based on clinical profile of an early-onset neurodegenerative disorder and characteristic MRI findings, the possibility of neurodegeneration with brain iron accumulation (NBIA) was considered. To confirm the diagnosis, next generation sequencing (NGS)-based targeted clinical exome analysis was performed. The patient was detected to have a novel homozygous splice donor site likely-pathogenic variant at position c.1039+2T>G in the *FA2H* gene (NM_024306.4) confirming the diagnosis of Fatty acid hydroxylase-associated neurodegeneration (FAHN). Another phenotype associated with *FA2H* is Spastic paraplegia type 35. However, this phenotype is now considered as a part of the spectrum of FAHN. The family was counselled regarding the genetic basis of the disorder, lack of specific treatment, risk of recurrence in their future pregnancies and availability of prenatal diagnosis.

The variant, c.1039+2T>G affects GT donor splice site of exon 6 of the *FA2H* gene (NM_024306.4). It has not been reported in both the 1000 genomes and ExAC databases. The *in silico* prediction of the variant was damaging or deleterious by various online predictive tools. The reference region was conserved across species. Analysis of this variant with the help of "Human splicing finder" revealed broken GT donor splice site due to this change.

Discussion

The *FA2H* gene codes for the enzyme, fatty acid 2-hydroxylase enzyme which catalyses hydroxylation at position 2 of N-acyl chain of ceramide moiety which is an important component of the myelin sheath (Gregory et al,2018; Schneider et al., 2013). Pathogenic variants in this gene have been found to be associated with demyelinating leukodystro-

phy (HSP35) and the NBIA - FAHN. The exact cause of disturbed iron metabolism and abnormal accumulation in the basal ganglia in FAHN is still not clear. A reasonable hypothesis developed is that the pallidum being a high metabolic demand structure, is vulnerable to subacute oxidative stress from mitochondrial dysfunction caused by intrinsic or extrinsic factors [Hayflick et al; 2014], and that the iron accumulation is triggered by the apoptotic cascade or cellular damage [Kruer et al., 2017]. The iron discarded from demyelination is not able to be re-used and hence gets accumulated in these structures because of specific predilection (Schneider et al., 2013).

Fatty acid hydroxylase-associated neurodegeneration (FAHN) is a subtype of NBIA (Gregory et al., 2018). It is a rare, autosomal recessive disorder, and till date a total of just over 50 individuals of diverse ethnicity with FAHN have been described (Kruer et al., 2017; Mari et al., 2018). The disorder can be recognised by the characteristic features of childhood-onset progressive spastic paraplegia that progresses to tetraparesis, or focal dystonia, ataxia, dysarthria, intellectual decline, and optic atrophy, accompanied by iron deposition in the brain. MRI studies demonstrate bilateral globus pallidus T2 hypointensity, consistent with iron deposition, prominent pontocerebellar atrophy, mild cortical atrophy, white matter lesions and corpus callosum thinning with variability among patients. Spastic paraplegia 35 is now considered as a part of the same disorder. Disease progression in FAHN is intermittent and there may be a period of clinical stability. Although premature death often occurs in the third or fourth decade secondary to a combination of nutrition-related immunodeficiency and respiratory compromise, life span is variable and genotype-phenotype correlation is difficult to make.

NBIA is an important group of neurodegenerative disorders to be identified in childhood, because of the recognisable clinical and radiological features. The common features in the group include a history of regression of milestones after a variable period of normal development, spasticity or extrapyramidal symptoms, and a variable neuroradiological picture. Differential diagnosis in this clinical setting include white matter disorders such as Metachromatic leukodystrophy, Krabbe disease, Pelizaeus- Merzbacher disease, Adrenoleukodystrophy, Juvenile Huntington disease, Friedreich's ataxia and Hereditary Spastic Paraplegias.

MRI of the brain typically shows hypo-intensities in the globus pallidus, substantia nigra and other basal ganglia structures. Cerebellar atrophy and visual impairment secondary to optic atrophy is not present in Pantothenate kinase-associated neurodegeneration (PKAN). The typical MRI features are: 'eye of the tiger' sign in PKAN; cerebellar atrophy in PLA2G6-associated neurodegeneration (PLAN) and Aceruloplasminemia; T2 -weighted signal hyperintensity with a central band of hypointensity in the substantia nigra in Beta-propeller Protein-Associated Neurodegeneration (BPAN); and cerebral, cerebellar and brain stem atrophy in Kufor-Rakeb syndrome (Gregory et al., 2018). Differential diagnosis for basal ganglia hypointensities include multiple sclerosis, Juvenile Huntington disease and Fahr disease. The age of onset of these disorders is however much later than for NBIAs.

The diagnostic yield of single gene disorders, including NBIA has increased greatly over the years owing to NGS technology. We previously described one case of *PLA2G6* -related neuronal degeneration using this technology (Goyal et al., 2015). Another study reported mutations in 15 families with PLAN using Sanger sequencing method from India (Kapoor et al., 2016). Whole genome sequencing technology offers even better diagnostic yield as it covers not only the coding regions but the entire genome including any copy number variation. Our case of FAHN adds to the spectrum of NBIA disorders that are seen in the Indian subcontinent.

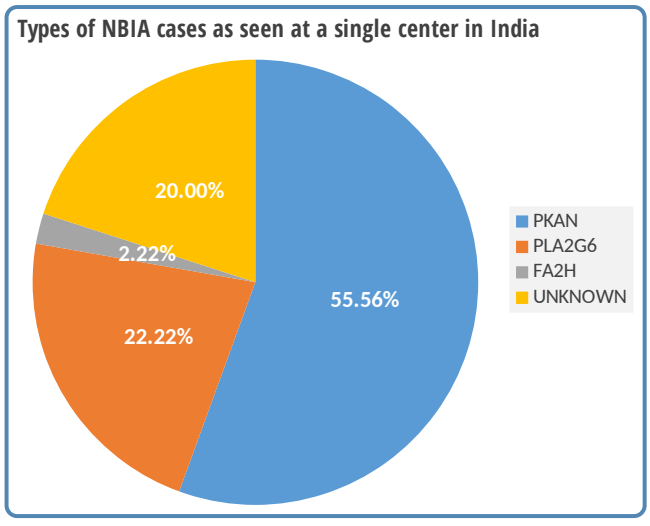

Figure 2 The distribution of cases of NBIA types seen over last 15 years at Institute of Genetics and Genomics Sir Ganga Ram Hospital New Delhi

In our own experience from the genetic clinic at Sir Ganga Ram Hospital of the last 15 years or so (Figure 2), from approximately 45 cases diagnosed with NBIA using clinical, neuroradiological and molecular methods, PKAN remains the most frequent followed by PLAN, with FAHN now adding a new type. Our findings are in agreement with those of others from various parts of the world. Our case emphasizes the importance of thorough clinical examination and the use of advanced molecular techniques like next generation sequencing in the accurate diagnosis of these rare disorders.

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