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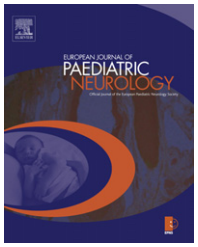

Case study

Juvenile parkinsonism associated with heterozygous frameshift *ATP13A2* gene mutation

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ABSTRACT

We report a case of levodopa-responsive juvenile parkinsonism (JP) associated with a heterozygous *ATP13A2* gene frameshift mutation. The clinical phenotype of our case is more severe when compared with other published reports of symptomatic heterozygous *ATP13A2* mutation carriers. To our knowledge, this is the youngest reported patient with JP associated with a heterozygous *ATP13A2* mutation. Our findings expand the clinical phenotypic spectrum of JP associated with heterozygous *ATP13A2* mutation.

1. Introduction

Juvenile parkinsonism (JP) is a term used to describe patients with parkinsonism with an onset under 21 years of age, whereas early-onset parkinsonism (EOP) refers to those with a disease onset between ages 21 and 40 years.[1–3] Mutations in recessively inherited genes are common causes of both JP and EOP.[1,4] In general, the younger the age of onset of parkinsonism, the more likely the patient has an inherited form of parkinsonism.[4] The most common cause of inherited JP and EOP are genetic *Parkin* mutations located on Chromosome 6q.[1,5]

The majority of levodopa-responsive JP patients without multi-systemic involvement are due to a genetic cause that follows an autosomal recessive (AR) mode of inheritance.[1,4] To date, 4 genes responsible for AR JP have been identified (*Parkin/PARK2, PINK1/PARK6, DJ-1/PARK7* and *ATP13A2/PARK9*) [4,6,7]. Interestingly all 4 of these genes are linked to mitochondrial dysfunction and oxidative stress suggesting a common pathway of pathogenesis.[6]

Homozygous or compound heterozygous *ATP13A2* gene mutations have been shown to cause AR JP and EOP associated with atypical features including dementia, pyramidal degeneration or supranuclear gaze paresis (Kufor-Rakeb syndrome

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(KRS)).[8–12] Recent publications have highlighted that JP and EOP patients with heterozygous mutations in the *ATP13A2* gene have a milder clinical presentation compared with the typical clinical phenotype of KRS.[13–16] These studies expand the clinical heterogeneity associated with *ATP13A2* mutations and suggest that heterozygous *ATP13A2* mutations may be associated with JP and EOP.

Due to the phenotypic heterogeneity associated with *ATP13A2* mutations, several authors have advocated screening for the *ATP13A2* gene in patients with EOP and in levodopa-responsive JP particularly with pyramidal signs or clinical features of KRS [13,17]. We now describe a case report of levodopa-responsive JP without additional clinical features of KRS associated with a novel heterozygous frameshift (truncating) mutation in the *ATP13A2* gene.

2. Case report

An 11-year old Lithuanian boy first presented at 6 years of age with a 1-year history of dysarthria, progressive upper limb postural tremor, dystonia of upper limbs associated with a diurnal variation. He did not have any systemic involvement. His symptoms resolved with levodopa (250 mg/day) treatment in Lithuania. At 7 years of age, the family then moved to UK and he was transferred to our care.

After 1½ years of good response to levodopa, he developed progressive motor fluctuations consisting of predominant bulbar symptoms and bradykinesia with rigidity. He subsequently required an increase of the levodopa dose up to 550 mg/day and adjunctive dopamine agonist treatment. This was associated with intermittent peak dose dyskinesia (See Table 1 for summary of clinical features of index case). Due to his increasing requirements of levodopa, further investigations were performed (See Table 2 for list of investigations performed).

Table 1 – Clinical features of patient.

Onset age (years)	5
Duration (years)	6
Family history of Parkinsonism	Negative
First symptoms	Dysarthria, dystonia of upper limbs
Asymmetric onset	Yes
Bradykinesia	Present (right > left)
Rigidity	Present bilaterally
Tremor	Present bilaterally with distal predominance (postural > at rest)
Postural instability	Mild
L-dopa response	Excellent
Motor fluctuations	Yes
Dyskinesias	Yes (prominent peak dose hyperkinesia)
Other motor features	Dysarthria and dysphagia becoming more pronounced in OFF-state
Psychiatric features	No
Autonomic features	No
Supranuclear gaze paresis	No
Babinski sign	Negative
Pyramidal neurological signs	No
Dementia	No
UPDRS (motor score)	OFF: 70, ON: 22
UPDRS (total score)	OFF: 125, ON: 39
Hoehn and Yahr stage	OFF: 5, ON: 2.5
Tinetti	OFF: 0, ON: 25

Table 2 – Laboratory investigations performed on patient (all normal except the tests highlighted bold with *).

Site	Test Performed
Brain	MRI
	[^{123}I] SPECT/DAT scan *
Peripheral nerve	Nerve conduction
CSF	Protein, cell count, glucose, lactate, bacterial culture Neurotransmitters
Plasma serum	FBC, clotting, U + E, LFT, Ca, Mg, CRP, thyroid function test
	Blood film looking for acanthocytes
	Ammonia, lactate, amino acid, very long chain fatty acid, angiotensin converting enzyme, white cell enzyme analysis
	Copper, caeruloplasmin
	Autoantibodies, autoimmune screen, lupus anticoagulant, rheumatoid factor, ASOT, anti-DNAse B
Blood DNA	DNA sequencing SCA 2 and SCA 3 genes
	DNA sequencing *ATP1A3* gene (Rapid onset-dystonia parkinsonism)
	DNA sequencing of entire coding region and MLPA analysis of all coding exons of *Parkin/PARK2*, *PINK1/PARK6* and *DJ-1/PARK7* genes
	DNA sequencing of entire coding region including highly conserved exon-intron splice junctions and MLPA analysis of exons 2, 9, 14 and 28 of *ATP13A2/PARK9* gene *
Urine	Organic acid chromatography, 24 h urine copper excretion

MRI, magnetic resonance imaging; SPECT, Single photon emission computed tomography; DAT, dopamine transporter; FBC, full blood count; U + E, urea and electrolytes; LFT, liver function tests; Ca, calcium; Mg, magnesium; CRP, C-reactive protein; ASOT, anti-streptolysin titre; anti-DNAse B, anti-DNAse B (streptococcal) antibodies; SCA, spinocerebellar ataxia.

Brain MRI scan was normal. There was no evidence of brain iron accumulation (on T2* gradient echo sequence), cerebral or caudate atrophy. Sural nerve biopsy was not performed.

DNA sequencing and MLPA analysis of *Parkin/PARK2*, *PINK1/PARK6* and *DJ-1/PARK7* genes were normal. DNA sequencing of entire coding region including highly conserved exon-intron splice junctions and MLPA analysis of exons 2, 9, 14 and 28 of *ATP13A2/PARK9* gene identified a heterozygous novel deletion of 13bp in exon 12 of *ATP13A2* gene that created a frameshift truncating mutation starting at codon Arg 370 resulting in an STOP codon 21 positions downstream (Fig. 1A). Both parents were also tested for this *ATP13A2* gene mutation. This heterozygous *ATP13A2* gene mutation was present in his clinically unaffected father (Fig. 1A) and was absent in his mother.

The region of the frameshift is highly conserved at the protein level (Fig. 1B) and is located in 3 different protein domains (ATPase P-type transporter, ATPase associated region and ATPase of unknown pump specificity Type V). Data of this mutation was obtained from Alamut software

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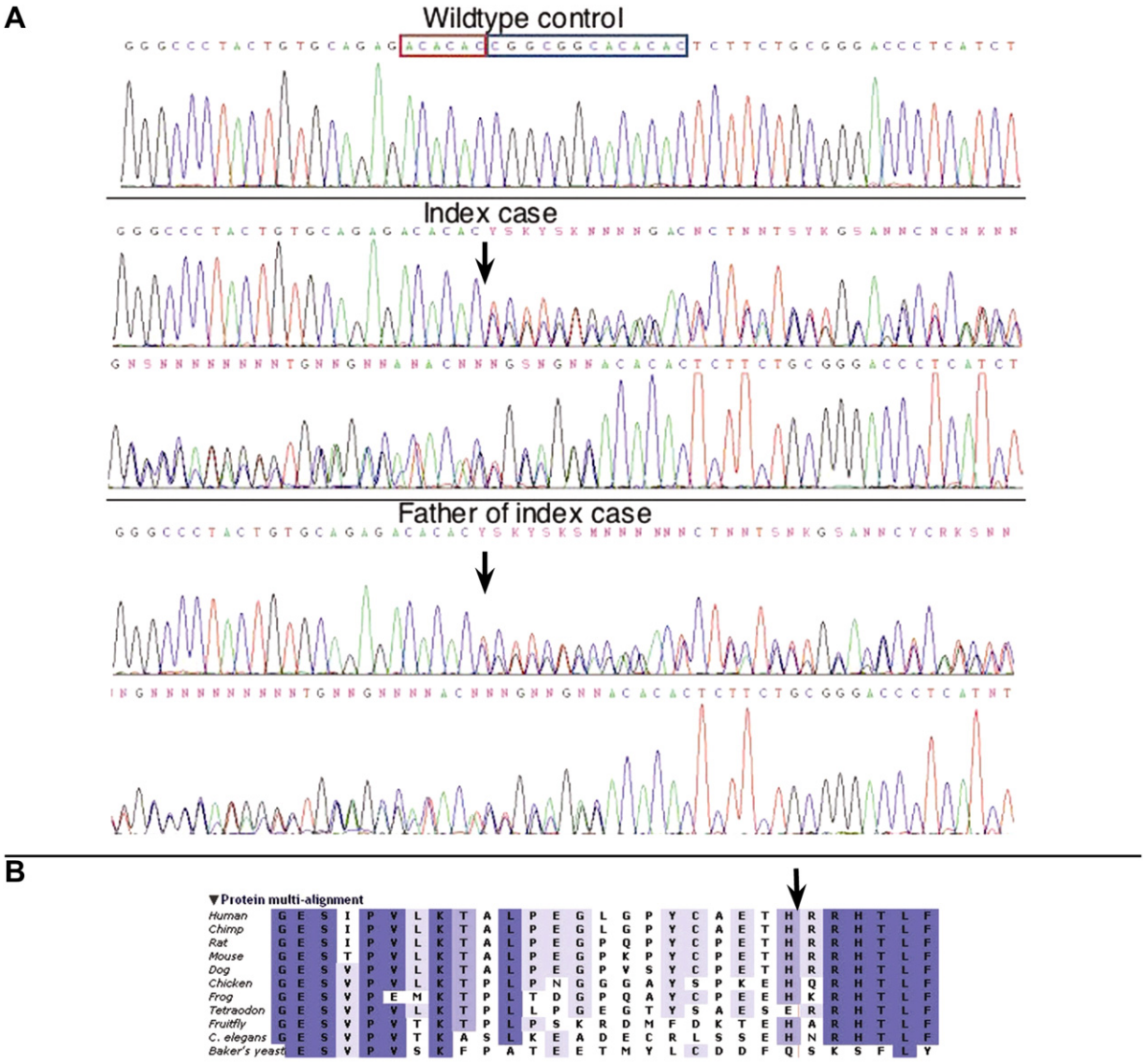

Fig. 1 – (A) Electropherograms of fragments of the *ATP13A2* genomic sequence. The position of the mutation identified in both index case and father of index case is indicated. The corresponding sequence from unrelated healthy control is provided as a reference (B) Conservation of the *ATP13A2* protein residues targeted by the mutation identified in patient. The closest homologues of the *ATP13A2* protein were aligned using the program Alamut. GenBank or ENSEMBL accession numbers are as follows: NP_071372.1 (ATPase type 13A2, Homo sapiens); XP_513111.2 (ATPase type 13A2, Chimp); NP_001166903.1 (ATPase type 13A2, Rat); NP_083373.2 (ATPase type 13A2, Mouse); ENSCAFP00000023307 (ATPase type 13A2, Dog); XP_422709.2 (ATPase type 13A2, chicken); ENSXETP00000029428 (ATPase type 13A2, frog); CG32000 (ATPase 13A2, fruitfly); NP_001024768.1 (ATPase type 13A2, c elegans).

programme (www.interactive-biosoftware.com). This mutation has not been seen in over 200 healthy European controls. The mutations were numbered from the A of the ATG-translation initiation codon, and the consequences of mutations at the protein level were predicted according to the *ATP13A2* mRNA sequence (GenBank accession number NM_022089.1) and protein sequence (accession number NP_071372.1).

kHis [^{123}I] SPECT/dopamine transporter (DAT) scan showed marked symmetrical reduction of activity in lentiform nuclei bilaterally consistent with his clinical symptoms (Fig. 2). Based on his clinical features supported by his DAT scan findings a diagnosis of JP was made. He is currently being considered for deep brain (pallidal) stimulation. His parents (father 36 years old and mother 37 years old) are non-consanguineous and clinically unaffected. His sibling sister of 17 years old is also clinically unaffected. There is no significant family history.

3. Discussion

To our knowledge, this is the youngest published case report of JP associated with a heterozygous mutation in the *ATP13A2* gene. The [^{123}I] SPECT scan in our patient showed marked

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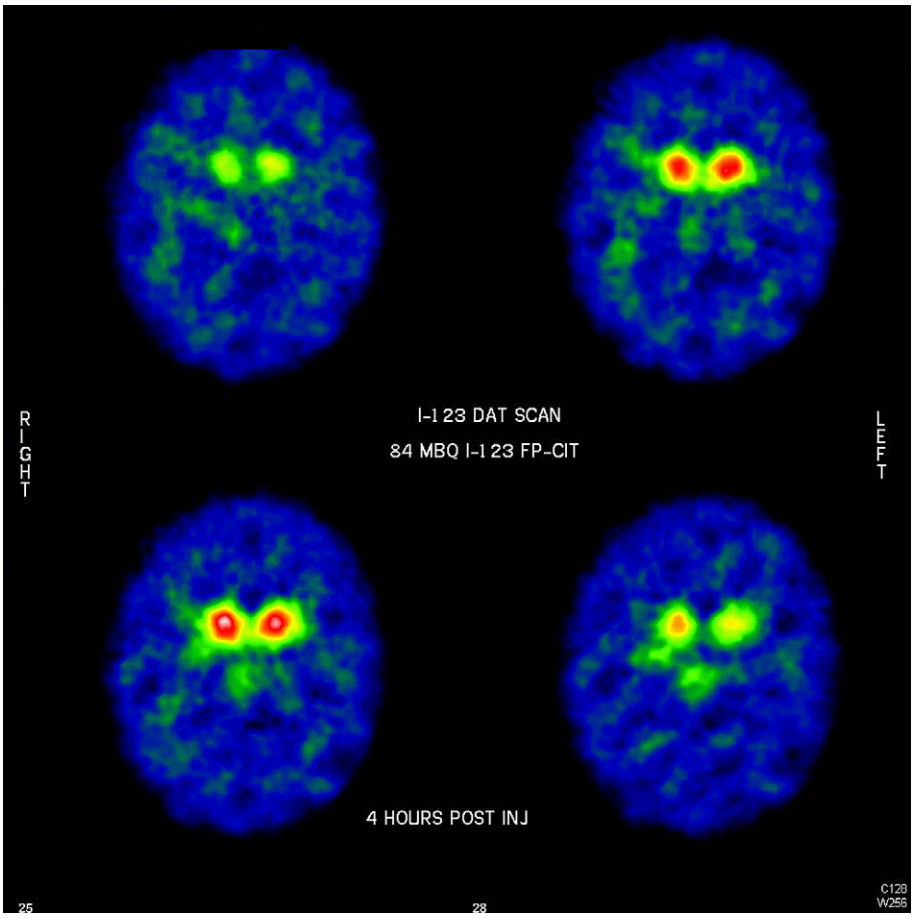

Fig. 2 – [^{123}I] SPECT scan of index case showing uptake of [^{123}I] in head of caudate nucleus bilaterally but marked symmetrical reduction of activity in lentiform nuclei bilaterally indicating diminished presynaptic dopamine transporter activity bilaterally.

symmetrical reduction of activity in lentiform nuclei bilaterally indicating presynaptic dopaminergic dysfunction in the nigrostriatal system. This is consistent with other published SPECT and PET findings in *Parkin, PINK1* and homozygous *ATP13A2* mutations.[5,14,18]

Homozygous or compound heterozygous *ATP13A2* gene mutations have recently been discovered to be the causative gene of the recessively inherited PARK9 also known as KRS.[9] The *ATP13A2* gene contains 29 exons encoding a large 1180 amino acids transmembrane lysosomal P-type ATPase protein.[11] The function and substrate specificity of this protein remain unknown, however the *ATP13A2* mRNA is highly expressed in brain, particularly in susbstantia nigra, and appears to be upregulated in the brain of patients with common late-onset idiopathic PD.[11] In-vitro models have shown that the *ATP13A2* mutated proteins were retained in the endoplasmic reticulum resulting in suspected overload of retained protein.[11]

To date, there are 10 published patients with potentially pathological heterozygous *ATP13A2* gene mutations of which 9 patients are reported to be associated with EOP or JP (age of onset between 20 and 40 years old).[13–16] These findings raise the possibility that heterozygous *ATP13A2* mutations might be aetiologically relevant in these patients and possibly act as a susceptibility risk factor. At present as mutations in *ATP13A2* are uncommon, the pathogenicity of heterozygous *ATP13A2* mutations is still uncertain.

The clinical phenotype of our patient appears to be worse than the previously reported symptomatic heterozygous *ATP13A2* mutation carriers.[13–16] Our patient is the youngest patient at disease onset (5 years old) and also displays a more rapid disease progression. After 6 years from first presentation, he now has significantly disabling dysarthria, dysphagia needing gastrostomy feeds and he is in an OFF-state in over 50% of the day. Our patient is however clinically distinct from KRS which is associated with homozygous or compound heterozygous *ATP13A2* mutations.

Determining the role of the heterozygous frameshift truncating mutation seen in our patient with JP is not easy but we believe that this mutation might be relevant to his disorder. This novel mutation results in a truncated protein with loss of about 2/3 of the protein and disrupts an E1-E2 ATPase domain. Thus, it is likely that this mutation has a biological consequence. Data from available international genetic databanks and theoretical prediction genetic programmes like PolyPhen and SIFT software have labelled that this deletion as pathological and of biological significance. Furthermore, this mutation has not been identified in over 200 control chromosomes indicating that this is not a common

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polymorphism. To draw more definitive conclusions about the pathogenicity of this mutation, *in vivo* or *in vitro* functional assays are required.

Whilst we have only detected a single mutation in our case, we could have missed the second mutation on the promoter or regulatory regions, or large genomic rearrangements that are not detectable on standard sequencing methods. Alternatively, it is also possible that the heterozygous frameshift mutation may act in a dominant-negative fashion contributing to the development of JP. We acknowledge that the patient's father who is clinically asymptomatic also carries the same heterozygous frameshift truncating mutation. It is however possible that the father may have not manifested clinical symptoms of PD yet or is a non-manifesting carrier.

PD is often associated with complex interactions among genes, epigenetic, and environmental factors.[19] Cumulative genetic and environmental factors of greater severity can result in disease onset at an earlier age. In parkinsonism, the distinctions between the mendelian inheritance patterns are less clear than the conventional mendelian classification. Single heterozygous mutations thought to be recessive (eg: *Parkin* and *PINK1* heterozygous mutations) can act as a susceptibility factor for parkinsonism and mimic a dominant mode of inheritance.[19,20] These heterozygous mutations may also act as disease modifiers affecting the penetrance, age of onset, severity and disease progression.

4. Conclusion

Our findings expand the clinical phenotypic spectrum of JP associated with heterozygous *ATP13A2* gene mutations. It suggests that heterozygous *ATP13A2* mutations may be a significant factor in the development of JP. This needs to be explored further in large-scale studies. It also highlights that more widespread screening of all cases of levodopa-responsive JP is warranted. Further functional studies are necessary to clarify the pathogenicity of this mutation.

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