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## Analysis of *ATP13A2* in large neurodegeneration with brain iron accumulation (NBIA) and dystonia-parkinsonism cohorts

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### Abstract

Several causative genes have been identified for both dystonia-parkinsonism and neurodegeneration with brain iron accumulation (NBIA), yet many patients do not have mutations in any of the known genes. Mutations in the *ATP13A2* lead to Kufor Rakeb disease, a form of autosomal recessive juvenile parkinsonism that also features oromandibular dystonia. More recently, evidence of iron deposition in the caudate and putamen have been reported in patients with *ATP13A2* mutations. We set out to determine the frequency of *ATP13A2* mutations in cohorts of idiopathic NBIA and dystonia-parkinsonism. We screened for large deletions using whole genome arrays, and sequenced the entire coding region in 92 cases of NBIA and 76 cases of dystonia-parkinsonism. A number of coding and non-coding sequence variants were identified in a heterozygous state, but none were predicted to be pathogenic based on *in silico* analyses. Our results indicate that *ATP13A2* mutations are a rare cause of both NBIA and dystonia-parkinsonism.

### Keywords

Neurodegeneration; Iron; NBIA; Kufor Rakeb; *ATP13A2*; Dystonia; Parkinsonism

## 1. Introduction

Although several genes have been identified that may lead to dystonia-parkinsonism [14], many cases remain idiopathic despite extensive workup. Similarly, many patients with neurodegeneration with brain iron accumulation (NBIA) lack mutations in any of the known genes [10].

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Kufor Rakeb disease (KRD) is an autosomal recessive pallido-pyramidal syndrome [11] caused by mutations in the lysosomal type 5 ATPase *ATP13A2* [13]. The parkinsonism in KRD is typically responsive to levodopa, at least initially, while dystonic orofacial spasms are also frequent in the disease [12].

Schneider and colleagues recently reported a case with homozygous mutations in *ATP13A2*, clinical parkinsonism, and T2\*MRI evidence of iron deposition [15]. Subsequently, at least one additional case with evidence of iron deposition has been identified [1], although most patients with *ATP13A2* mutations do not have identifiable brain iron deposits despite being clinically symptomatic [1,4]. Given the clinical and neuroimaging overlap between KRD, dystonia-parkinsonism, and NBIA, we sought to determine if patients with idiopathic forms of NBIA or dystonia-parkinsonism harbored mutations in *ATP13A2*.

## 2. Materials and methods

### 2.1. Subjects

Approval for this study as part of an ongoing study of NBIA and dystonia-parkinsonism was obtained through the Oregon Health & Science University institutional review board (US) or the ethics committee of the National Hospital for Neurology & Neurosurgery (UK). Patients were referred for study by treating physicians because of clinical dystonia-parkinsonism or NBIA. This population was diverse and included patients from five continents. The majority of these patients represented sporadic, non-consanguineous cases contributed by referring physicians outside of both institutions. A diagnosis of idiopathic NBIA was made if probands had evidence of iron deposition in the basal ganglia (Fig. 1) without identifiable mutations in *PANK2*, *PLA2G6*, *c19orf12*, or *FA2H*. *FTL* and *ACP* sequence analysis was performed in select cases with evidence of iron deposition in the caudate, thalamus, or dentate nuclei. Dystonia-parkinsonism cases presented without mutations in *PANK2* or *PLA2G6*. Written informed consent was collected for all participants. Whole blood was collected in citrate ACD or EDTA collection tubes.

### 2.2. Genotyping and sequencing

Genomic DNA was extracted from whole blood or EBV-transformed lymphoblast cultures prepared from the original blood sample using standard methods. Mutation analysis for known causes of NBIA or dystonia-parkinsonism was negative in cases selected for inclusion in the current study. Twenty-six patients had array-based genomic analyses (Illumina HumanCNV370-Quad or CytoSNP 12 Beadchips) performed per the manufacturer's instructions which excluded duplications, deletions, and insertions larger than ~25–30 kb. Genomic copy number variants were detected using the Genome Studio CNV plugin ([http://www.illumina.com/software/illumina\\_connect.ilmn](http://www.illumina.com/software/illumina_connect.ilmn)). DNA sequence analysis was then performed using Sanger sequencing. Primers were designed to amplify all 29 coding exons and at least 30 bp of the intron–exon boundary of *ATP13A2* (sequences available upon request) using PrimerZ (<http://genepipe.ngc.sinica.edu.tw/primerz/beginDesign.do>) or ExonPrimer (<http://ihg.gsf.de/ihg/ExonPrimer.html>). Polymerase chain reaction (PCR) amplification was performed in a total volume of 12 ml using 10–20 ng of genomic DNA, 10 pmol transcript-specific forward and reverse primers, and 10 ml FastStart PCR Master Mix (Roche). Each purified product was sequenced in forward and reverse directions using BigDye-terminator version 3.1 (Applied Biosystems). Purified sequences were run on an ABI3730XP automated sequencer per the manufacturer's protocol (Applied Biosystems). Sequence data were analyzed using Sequencher version 4.1.4 (GeneCodes). Sequence variants were identified via comparison to reference sequence (NM 022089.2) using Ensembl ([www.ensembl.org](http://www.ensembl.org)) and Mutalyzer (Wildeman et al., 2008). *In silico*

algorithms (SIFT (<http://sift.jcvi.org/>) and PolyPhen (<http://genetics.bwh.harvard.edu/pph/>) were used to interrogate any sequence variants identified.

### 3. Results

Microarray analysis demonstrated no deletions or duplications within 1 Mb of the *ATP13A2* physical position (GRCh37/hg19 chr1:17,312,453–17,338,423) in any of the samples analyzed. Sequence analysis revealed several coding and noncoding sequence variants (Tables 1 and 2). Several variants (rs2076603, rs3738815, rs9435662, rs3170740) represented well-characterized high-frequency SNPs, present in 20% of cases, and were not considered further. Although we identified several sequence variants that had not been previously identified, none of these variants were predicted to be deleterious using *in silico* algorithms, and none were found in a homozygous or compound heterozygous state, indicating that they likely represent benign polymorphisms.

#### 3.1. Analysis of sequence variants

**3.1.1. Dystonia-parkinsonism**—We observed a c.881G>A polymorphism within exon 10 (p.R294Q) in a single patient with dystonia-parkinsonism and ataxia that began at age 23. This patient had T2 hyperintense signal changes in the basal ganglia on MRI. Although this variant was predicted to be ‘possibly damaging’ by one of the *in silico* algorithms, it was predicted to be tolerated by another, and it is published in dbSNP as rs56367069. Multiple sequence alignment demonstrated sequence conservation across mammalian species, but variability in lower organisms. This variant has previously been observed in neurologically normal individuals [16], indicating it is not pathogenic.

Also within exon 10, a novel sequence change (c.898C>A) was observed in 4 patients. Patient 1 presented at age 40 with personality change, cognitive decline, dystonia-parkinsonism, myoclonus and apraxia, and T2 hyperintensity in the basal ganglia on MRI. Patient 2 developed dystonia-parkinsonism, chorea, ataxia, and cognitive decline at age 36. Patient 3 exhibited extrapyramidal features, ataxia, and cognitive decline beginning in the late teens associated with basal ganglia T2 hyperintensity. Patient 4 developed ataxia, dystonia, and cognitive decline with frontal release signs and mutism at age 32; MRI demonstrated ex vacuo ventriculomegaly and diffuse T2 white matter hyperintensity. Predicted to lead to a heterozygous p.P300T substitution within the ATPase-associated domain, the c.898C>A sequence variant was considered unlikely to be deleterious based on *in silico* analysis. Sequence identity was not conserved between species.

In a patient with onset of dystonia-parkinsonism and cognitive decline at age 30, a single c.1043C>T heterozygous variant was identified in exon 13. This sequence change led to a p.R414W substitution that also fell within the ATPase-associated domain. *In silico* modeling indicated that this variant was unlikely to be pathogenic.

**3.1.2. NBIA**—A c.472G>A variant was detected within exon 5 in a single NBIA patient, who presented w/extrapyramidal symptoms and hypointensity of the globus pallidus and substantia nigra. This variant has previously been assigned SNP ID rs113643181. While the ensuing p.G158R heterozygous variant was predicted to affect protein function by one algorithm, it was predicted to be a benign change by the other. This residue is not conserved across species, and occurs outside of both the P-type ATPase and transmembrane domains of the protein. This sequence change thus most likely represents a benign variant.

The p.R294Q variant was detected in a heterozygous state in three NBIA patients, in addition to being detected in dystonia-parkinsonism.

In a patient who developed axial rigidity, resting tremor, myoclonic jerks, and foot drop at age 16 (along with neuroimaging indicating iron deposition), a heterozygous c.1109G>A variant in exon 12 was identified. The resultant p.R370Q residue change was predicted to be benign, and in fact, a glutamine is found at this site in lieu of arginine in the *ATP13A2* homolog *Ypk9* in *S. cerevisiae*.

In a patient with dystonia-parkinsonism, pyramidal tract signs, and T2 hypointensity of the basal ganglia, 2 heterozygous sequence changes were noted in exon 29 (c.3365C>T and c.3472G>A). These variants were predicted to lead to p.P1122L (rs15786) and p.V1158M (rs76298930), respectively. In addition to being previously described in unaffected individuals, both variants were predicted to represent benign polymorphisms.

Additional novel sequence changes (c.132A>G, c.1536C>T, and c.2439C>T) led to synonymous amino acid residues and are thus unlikely to be of consequence.

#### 4. Discussion

Considerable phenotypic overlap exists between subtypes of NBIA and dystonia-parkinsonism. In particular, mutations in *PLA2G6* have been associated with surprisingly diverse phenotypes that include psychomotor regression in infancy with death in early childhood at one end of the spectrum and adult-onset parkinsonism-dystonia at the other. Patients with mutations in *PLA2G6* variably exhibit evidence of iron deposition in the globus pallidus and substantia nigra [10], perhaps related to differential effects of mutations on the enzyme's catalytic activity toward phospholipids and lysophospholipids [7].

Given that select patients with mutations in *ATP13A2* developed MRI evidence of iron deposition in the basal ganglia and that both dystonia and parkinsonism are features of KRD, we sought to determine the frequency of *ATP13A2* mutations in large cohorts of patients with dystonia-parkinsonism with or without brain iron deposition of unknown cause. It is worth mention that although homozygous or compound heterozygous mutations are known to lead to the complicated neurological phenotype of Kufor Rakeb disease, heterozygous *ATP13A2* mutations have been implicated in cases of isolated parkinsonism, often of early-onset [3,5,9]. To date, *ATP13A2* mutations have not been detected in patient cohorts with typical-onset sporadic Parkinson's disease [6,16].

Our results indicate that *ATP13A2* mutations are rare in cases of both dystonia-parkinsonism and NBIA ascertained based on clinical and neuroimaging findings. These results are not surprising given that few cases of most single gene forms of these disorders have been identified worldwide [14]. These findings may help guide the clinical evaluation of parkinsonian disorders.

#### 5. Conclusions

Mutations in *ATP13A2* are associated with a complex phenotype. The nosology of this disorder is further complicated by the recent finding that both Tibetan terriers and a human family with brain accumulation of ceroid lipofuscin suggestive of Kufs' disease bear mutations in *ATP13A2* [2,8] indicating phenotypic overlap with the neuronal ceroid lipofuscinoses. The current lack of knowledge related to the function of *ATP13A2* and the fact that no postmortem Kufor Rakeb samples have been reported suggests that other approaches, such as the use of mouse models, will likely be necessary to elucidate the pathophysiologic basis of this unusual parkinsonian disorder.

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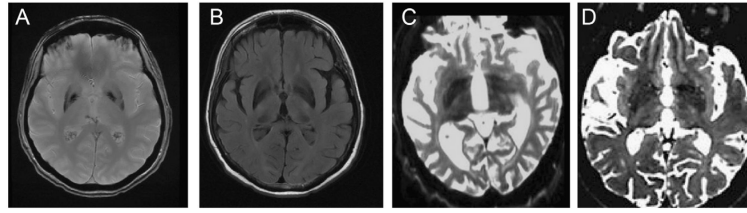
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**HIGHLIGHTS**

- ▶ Mutations in *ATP13A2* may lead to complex, overlapping neurodegenerative phenotypes.
- ▶ Dystonia, parkinsonism, brain iron deposition, and ceroid lipofuscinosis have been described.
- ▶ A cause is not able to be identified for many patients with dystonia-parkinsonism (D-PD) and NBIA.
- ▶ We screened the largest assembled cohort to date of D-PD and NBIA patients for mutations in *ATP13A2*.
- ▶ *ATP13A2* mutations are at best a rare cause of both phenotypes.





**Fig. 1.** Neuroimaging features of idiopathic NBIA and Kufor Rakeb Disease. Axial T2\* (A) and FLAIR (B) images from a 68 year-old woman with cognitive decline and parkinsonism diagnosed with idiopathic NBIA. (C) and (D) represent axial T2-weighted images taken from patients with compound heterozygous mutations (c.3057delC/c.130615G → A) in *ATP13A2* (images C and D reprinted with permission from [1,15]).



**Table 1**

*ATP13A2* sequence variants identified in dystonia-parkinsonism (a) and NBIA (b) cases.

Location	Physical position <sup>a</sup>	SNP ID	Variant	Frequency	Interpretation
(a)					
Exon 10	chr1:17,326,767	rs56367069	c.881G>A	1/76 (1%)	p.R294Q(het) tolerated/possibly damaging
Exon 10	chr1:17,326,750		c.898C>A	4/76 (5%)	p.P300T (het) tolerated
Exon 13	chr1: 17,322,928		c.1043C>T	1/76 (1%)	p.R414W (het) tolerated
Location	Physical position <sup>a</sup>	SNP ID	Variant	Frequency <sup>b</sup>	Interpretation
(b)					
Exon 3	chr1:17,332,025		c.132A>G	1/92 (1%)	Synonymous coding
Exon 5	chr1:17,331,192	rs113643181	c.472G>A	1/92 (1%)	p.G158R (het) affect protein function/benign
Exon 10	chr1:17,326,540	rs56290406	c.1005C>T	1/92 (1%)	Synonymous coding
Exon 10	chr1:17,326,767	rs56367069	c.881G>A	3/92 (3%)	p.R294Q(het) tolerated/possibly damaging
Exon 12	chr1:17,323,601		c.1109G>A	1/92 (1%)	p. R370Q(het) benign
Exon 15	chr1:17,322,477		c.1536C>T	1/92 (1%)	Synonymous coding
Exon 22	chr1:17,316,472		c.2439C>T	1/92 (1%)	Synonymous coding
Exon 26	chr1:17,313,654	rs761421	c.2970G>A	2/92 (2%)	Synonymous coding
Exon 27	chr1:17,313,343	rs9435659	c.3192C>T	1/92 (1%)	Synonymous coding
Exon 28	chr1:17,313,021	rs115985012	c.3342C>T	2/92 (2%)	Synonymous coding
Exon 29	chr1:17,312,592	rs15786	c.3365C>T	1/92 (1%)	p.P1122L(het) benign
Exon 29	chr1:17,312,485	rs76298930	c.3472G>A	1/92 (1%)	p.V1158M (het) benign

<sup>a</sup>Physical position based upon RCh37.

<sup>b</sup>Present in either a heterozygous or homozygous state.

**Table 2***ATP13A2* sequence variants identified in NBIA cases.

Location	Physical position <sup>a</sup>	SNP ID	Variant	Frequency <sup>b</sup>	Interpretation
Exon 3	chr1:17,332,025		c.132A>G	1/92 (1%)	Synonymous coding
Exon 5	chr1:17,331,192	rs113643181	c.472G>A	1/92 (1%)	p.G158R (het) affect protein function/benign
Exon 10	chr1:17,326,540	rs56290406	c.1005C>T	1/92 (1%)	Synonymous coding
Exon 10	chr1:17,326,767	rs56367069	c.881G>A	3/92 (3%)	p.R294Q(het) tolerated/possibly damaging
Exon 12	chr1:17,323,601		c.1109G>A	1/92 (1%)	p.R370Q(het) benign
Exon 15	chr1:17,322,477		c.1536C>T	1/92 (1%)	Synonymous coding
Exon 22	chr1:17,316,472		c.2439C>T	1/92 (1%)	Synonymous coding
Exon 26	chr1:17,313,654	rs761421	c.2970G>A	2/92 (2%)	Synonymous coding
Exon 27	chr1:17,313,343	rs9435659	c.3192C>T	1/92 (1%)	Synonymous coding
Exon 28	chr1:17,313,021	rs115985012	c.3342C>T	2/92 (2%)	Synonymous coding
Exon 29	chr1:17,312,592	rs15786	c.3365C>T	1/92 (1%)	p.P1122L(het) benign
Exon 29	chr1:17,312,485	rs76298930	c.3472G>A	1/92 (1%)	p.V1158M (het) benign

<sup>a</sup>Physical position based upon RCh37.<sup>b</sup>Present in either a heterozygous or homozygous state.