Short Report

Novel mutation in ATP13A2 widens the spectrum of Kufor-Rakeb syndrome (PARK9)

Kufor-Rakeb syndrome (KRS) is a rare autosomal recessive inherited juvenile parkinsonian syndrome caused by mutations in ATP13A2. We describe six patients from a consanguineous Greenlandic Inuit family, homozygous for a novel frame-shift mutation in exon 22 of ATP13A2 (c.2473C>A, p.Leu825AsnfsX32). Disease onset varied from 10 to 29 years of age, the latest reported, and the clinical features were highly variable within a wide spectrum of an extrapyramidal–pyramidal syndrome with cognitive/psychiatric features. Ataxia was seen in two patients and axonal neuropathy in one, features not previously related to KRS. Dopamine transporter scans showed symmetrical, severely reduced uptake in striatum in two patients. Magnetic resonance imaging was without atrophy in one patient despite disease duration of 17 years, and cerebral and cerebellar atrophy was seen in another patient after 4 years of disease duration. The molecular pathogenic mechanisms of ATP13A2 mutations are discussed. The observation that the mutant transcript is not degraded by nonsense-mediated RNA decay and the fact that none of the eight heterozygous carriers from the family have KRS symptoms suggest that the mutant protein does not interfere and destroy the function of the wild-type ATP13A2 protein.

Conflicts of interest
The authors declare that they have no conflict of interest.
Novel ATP13A2 mutation widens KRS spectrum

A genome-wide single nucleotide polymorphism (SNP) microarray analysis (SNP6.0, Affymetrix, Santa Clara, CA, and AROS Applied Biotechnology, Aarhus, Denmark) was conducted for the affected individuals V-1 and V-9 and analyzed for loss of heterozygosity, copy number variations and homozygosity using the Chromosome Analysis Suite (Affymetrix). Exclusion analyses, including all family members, were performed for all homozygous regions using polymorphic sequence-tagged site (STS) markers and a 3-primer fluorescence labeling system (21). Oligonucleotides for polymerase chain reaction (PCR) and DNA sequencing of the ATP13A2 gene were constructed using Primer3 (22) (sequencing primer and STS marker sequences are available on request). Total RNA was isolated from whole blood using PAXgene Blood RNA Tubes and PAXgene Blood RNA Kit (Qiagen, Copenhagen, Denmark). Complementary DNA (cDNA) synthesis was carried out using SuperScript II Reverse Transcriptase according to the manufacturer’s protocol (Invitrogen, Taastrup, Denmark) and PCR was carried out using primers annealing to exon 21 and exon 23 and spanning the mutation site. The PCR products were separated by 2% agarose gel electrophoresis and subsequently Sanger sequenced for detection of mutation carriers. Co-segregation of the mutation in the family and detection of the mutation prevalence in the Inuit population was carried out using DdeI (New England Biolab, Ipswich, MA) for digestion of PCR products for exon 22 (Fig. 2b). Two-point logarithm of odds (LOD) score was calculated using the computer program LIPED (Likelihoods in PEDigrees) (23) with allele frequencies of 0.001 for the disease allele.

Material and methods

Several members of a large consanguineous family in Greenland have since 1979 been evaluated independently by different general practitioners and incoming neurologists who did not have a full overview of the family because of the complex family relations (Fig. 1). Clinical information was collected locally by the chief medical physician of the Mid Greenland Health Region and three affected family members were independently admitted to two different neurological departments in Copenhagen, Denmark (Fig. 1: patients V-1, V-3 and V-5), before the diagnosis of PARK9 was established.

Mutations in the genes HTT, ATN1, ATXN1, ATXN2, ATXN3, CACNA1A, ATXN7, TBP, TOR1A, GCH1, SGCE, FRDA, MAPT, PRGN, PS1, PS2, and APP were excluded by fragment analyses or direct sequencing. Exon rearrangements in SNCA, PRKN, PINK1, DJ1, LRRK2 (including p.Gly2019Ser) and ATP13A2 were excluded by multiplex ligation-dependent probe amplification (MLPA) (MRC-Holland, Amsterdam, the Netherlands).

Fig. 1. Pedigree of the consanguineous Inuit family with the Kufor-Rakeb disease. Affected individuals are marked with a filled symbol, a half-filled symbol represents individuals heterozygous for the mutation, and individuals having a symbol with a vertical black line represent inferred carriers. The genotype mut/mut represents the homozygous state for the mutation and wt/mut represents carriers. A solid line above the symbol marks individuals where DNA was available.
Fig. 2. (a) The DNA sequencing chromatograms represent the wild-type allele (wt/wt), the heterozygous carrier of the indel c.2473C>AA mutation (wt/mut), and the affected homozygous mutations carrier (mut/mut). The corresponding DNA sequence is shown beneath the chromatograms and the arrows denote the deleted wild-type cytosine and the inserted two adenosines in the indel mutation. (b) The DdeI enzyme recognized the wild-type but not the mutant c.2473C>AA allele and a digest of the exon 22 PCR product confirmed the mutation in the family. Individuals IV-7 and IV-8 are heterozygous carriers, and individuals IV-6, V-1, V-3, V-5 and V-9 are homozygous affected. Lane C represents a normal control individual. The wild-type allele fragments are 119, 81, 37 and 5 bp; the mutant allele fragments are 201 37 and 5 bp. U represents the uncut 242 bp PCR product. The PCR products were separated in 2% agarose and DNA was stained with ethidium bromide. (c) PCR amplicons using cDNA synthesized from RNA from blood as template and primers to ATP13A2 exon 21 and exon 23, respectively. The 161 bp ex21–23 PCR product spanning the mutation site can be detected both in the homozygous affected individuals V-3 and V-9 (mut/mut) as well as in two healthy (wt/wt) children of V-5 and in a control sample (wt/wt). The size marker is a 100 bp ladder. Both the homozygous mutation state and the wt/wt genotype were confirmed by DNA sequencing of the PCR products. (d) Schematic presentation of the wild-type and the truncated mutant ATP13A2 protein shows the shortened hydrolase domain and the missing transmembrane domains. The predicted protein structures were constructed using the program SMART (http://smart.embl-heidelberg.de/ and reference sequence NP_071372). (e) A graphic presentation of the 11 subunit transmembrane spanning ATP13A2 protein and the three intracellularly located functional domains shows the positions of known pathogenic frame-shift and missense mutations and putative pathogenic single nucleotide variants (Table 2). The domain structure was adapted from the predicted ATP13A2 protein structure using SMART http://smart.embl-heidelberg.de/ and the transmembrane structure was predicted using TMHMM (http://www.cbs.dtu.dk/services/TMHMM/ and reference protein NP_071372). The majority of the mutations are located in the intracellular functional domains.
Results

Clinical reports

Patient number IV-1 (female died at the age of 57)
She developed ‘tiredness’ in her legs, frequent falls, and intermittent diplopia at age 27. At age 40, she developed slurred speech and reduced sensibility in the left side of her body and subsequently slowly progressive tetraparesis with mutism. A brain computerised tomography (CT) showed cerebral atrophy at age 32 (Table 1).

Patient number IV-6 (45-year-old male)
He had onset at age 24 of progressing weakness of his right leg and intermittent diplopia. At age 28, he developed psychotic symptoms with auditory hallucinations and cognitive decline. He is presently mute, wheelchair bound, and demented. MRI of the brain showed global cerebral and cerebellar atrophy at age 28 (Table 1).

Patient number V-1 (30-year-old male)
Motor milestones were normal in early childhood until a near drowning accident at age 6. He had cardiac arrest and was resuscitated. Consequently, he was mentally changed and slow but did not have any parkinsonian symptoms until age 12, when he developed tremor of his left arm, drooling, retrocollis, hypophonic voice, and a slower and more unstable gait making him wheelchair bound. He experienced auditory and visual hallucinations and panic attacks. CT of the brain at 19 years was normal. Examination at age 29 revealed cognitive impairment, oligomimia with facial and tongue mini-myoclonus. He had vertical supranuclear gaze palsy, and the pursuits were jerky, while the saccades were normal. Cogwheel rigidity, moderate ataxia, and hyperactive tendon reflexes were present in the upper limbs. In the lower limbs, mild ataxia and cogwheel rigidity were found in addition to a moderate spastic paraparesis with hyperactive patellar reflexes and bilateral Babinski’s sign, whereas ankle reflexes were normal. Levodopa has a lasting moderate effect on his parkinsonism. MRI of the brain at age 29 revealed unspecific gliosis anterior to the left ventricle but no atrophy. A DAT scan, using \[^{123}I\]FP-CIT (N-\(\omega\)-fluoropropyl-2\(\beta\)-carbomethoxy-3\(\beta\)-(4-iodophenyl)nortropane) binding in striatum (Fig. 3b), and brain CT was normal (Table 1).

Patient number V-3 (23-year-old female)
She had onset at age 10 with bradykinesia and rigidity followed by progressive cognitive decline. CT scan of the brain at age 12 was normal. Treatment with levodopa had a moderate effect. At age 23, while on levodopa therapy, examination revealed normal function of cranial nerves, except bradykinesia of the tongue. She had normal tone but severe dyskinesias in the upper limbs and slight rigidity in the lower limbs. The gait was slow and stiff with slight propulsion. Without treatment, she had severe bradykinesia and moderate rigidity. No ataxia was found, and tendon reflexes and sensory findings were normal. She had no hallucinations.

A DAT scan showed symmetrical severely reduced \[^{123}I\]FP-CIT binding in striatum (Fig. 3b), and brain CT was normal (Table 1).

Patient number V-5 (32-year-old female)
She had onset of progressing weakness of the right leg, impaired balance, and memory disturbances at age 29, but no psychiatric symptoms. At age 31, neurological examination revealed difficulties in word finding, and she was perceived as depressed. She was unable to perform Luria’s hand test. She had facial mini-myoclonus below the eyes and periorally, most prominent in her tongue. The pursuits were normal, but saccades were slow and hypometric, and she had vertical supranuclear gaze palsy. The jaw jerk was brisk, and the snout and palmomentum reflexes were present. The tendon reflexes in the upper limbs were hyperactive. In the lower limbs, a mild weakness and decreased tone were found. The proximal tendon reflexes were brisk. The ankle reflexes were decreased, but Babinski’s sign was present bilaterally. She had striatal toes and pes cavus. There were normal sensory findings, but she had a slight truncal and a moderate limb ataxia. Her gait was broad based and unstable. At age 31, she scored 5 of 12 points in the Rivermead Behavioural Memory Test. MRI of the brain was slightly suspicious of enlarged ventricles and a gracile spinal cord. Electroneuropgraphy (ENG) showed decreased motor amplitudes and slightly prolonged F-wave latencies from the tibial and peroneal nerves on both sides. SomatoSensory Evoked Potentials (SSEPs) were indicative of a central affection of the somatosensory tracts from the lower limbs, and Magnetic Evoked Potentials (MEPs) were with a pronounced affection of the corticospinal tracts to the upper and lower limbs (Table 1).

Patient number V-9 (23-year-old female)
She had insidious onset at age 15 with bradykinesia and reduced speech production, mandibular
<table>
<thead>
<tr>
<th>Individual</th>
<th>IV-1</th>
<th>IV-6</th>
<th>V-1</th>
<th>V-3</th>
<th>V-5</th>
<th>V-9</th>
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<tr>
<td>Age at onset (years)</td>
<td>27</td>
<td>24</td>
<td>12</td>
<td>10</td>
<td>29</td>
<td>15</td>
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<tr>
<td>Initial symptom</td>
<td>Fatigue and falls</td>
<td>Weakness of right leg and diplopia</td>
<td>Tremor of left hand, salivation, and retrocollis</td>
<td>Cognitive and motor decline</td>
<td>Gait and balance problems – initially affecting right leg</td>
<td>Bradypodia, motor and cognitive decline</td>
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<td></td>
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<td>NA</td>
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<td>NA</td>
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<td>Ataxia</td>
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<td>Cognitive decline</td>
<td>Yes</td>
<td>Yes</td>
<td>Visual and auditory</td>
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<td>Probably</td>
<td>Yes</td>
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<td></td>
<td></td>
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<td>Brain CT</td>
<td>32 years: cerebral atrophy</td>
<td>ND</td>
<td>19 years: normal</td>
<td>12 years: normal</td>
<td>31 years: normal</td>
<td>22 years: normal</td>
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<tr>
<td>Brain MRI</td>
<td>ND</td>
<td>28 years: cerebral and cerebellar atrophy</td>
<td>29 years: small unspecific hyperintensity, no atrophy</td>
<td>ND</td>
<td>31 years: slightly enlarged ventricles and a gracile spinal cord</td>
<td>ND</td>
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<tr>
<td>DAT scans</td>
<td>ND</td>
<td>ND</td>
<td>Symmetrical severely reduced uptake in striatum</td>
<td>Symmetrical severely reduced uptake in striatum</td>
<td>ND</td>
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NA, not assessed; ND, not done.
Novel ATP13A2 mutation widens KRS spectrum

The mutant ATP13A2 c.2473C>AA gene (p.Leu825AsnfsX32) encodes an 857-amino acid-long truncated chimera protein carrying an extension of 31 amino acids at position p.Leu825 (Fig. 2c). The frame-shift results in deletion of 325 amino acids, including part the hydrolase domain and six C-terminally located transmembrane alpha-helixes (Fig. 2d).

Expression analysis of ATP13A2 in blood leukocytes from two affected and two non-affected individuals from the family, and one control individual, showed the mutant transcript in the affected and the wild-type transcript in the non-affected relatives and the control. Detection of the transcript in the affected excludes nonsense-mediated decay (NMD) of the ATP13A2 c.2473C>AA mutant RNA transcript. A subsequent study of the carrier status for the p.Leu825AsnfsX32 mutation was performed in 103 individuals from the isolated village. This study included immediate family members, extended relatives, and unrelated individuals, either non-affected or with a mild parkinsonian phenotype. A total of eight carriers, all related to the family, were identified. None of these had parkinsonism. More importantly, none of the individuals with the mild parkinsonian phenotype carried the mutation.

Discussion

Investigations of this Greenlandic Inuit family were started many years prior to the establishment of the KRS diagnosis. The clinical information and neurological examinations all lie temporally before the diagnosis of KRS, and hence are unbiased by the diagnosis. Age at onset varied from 10 to 29 years with symmetric or asymmetric motor symptoms and/or cognitive decline. The clinical features were highly variable within a wide spectrum of an extrapyramidal–pyramidal syndrome with cognitive/psychiatric features in all patients (Table 1). Three patients (IV-1, IV-6, and V-5) had a relatively late age at onset, at 27, 24 and 29 years, respectively, as compared with a range of 10–22 years in previously reported cases. A cardinal feature of KRS is parkinsonism, but V-5 did not have overt parkinsonian symptoms, possibly reflecting her short disease duration of 3 years, and she had no spasticity but hyperactive reflexes and Babinski’s sign. Cerebellar symptoms and signs were prominent: a feature not previously described in KRS and presumably unrelated to late onset of disease, as V-1 with onset at age 12 also had prominent ataxia. As expected, electrophysiological examination showed prolonged central conduction time in V-5; however, examination of the
peripheral nerves showed signs of an axonal neuropathy, which may be an unrecognized feature of KRS. Neurophysiological parameters including electromyography (EMG), nerve conduction studies (NCS) and SSEPs were normal in the patient reported by Schneider et al. (5), and more structured electrophysiological studies on KRS are needed to clarify a peripheral component.

Brain CT/MRI revealed global cerebral atrophy in two of six patients, but V-1 with disease duration of 17 years did not have any visible atrophy on MRI, whereas IV-6 showed global cerebral and cerebellar atrophy after disease duration of 4 years. There were no signs of iron deposition on the MRI of patients V-1 and V-5, but we did not have sensitive MRI sequences for assessment of iron deposition. On the basis of iron accumulation in the basal ganglia in one patient determined by MRI T2*, Schneider et al. proposed to classify KRS as Neurodegeneration with Brain Iron Accumulation (NBIA) type 3 (5). Iron accumulation was recently confirmed in one more patient by Brüggemann et al. (11). In contrast, both Santoro et al. (10) and Chien et al. (25) detected no brain iron accumulation on T2* MRI in patients with non-synonymous mutations in the ATP13A2 protein, suggesting that missense mutations lead to a phenotype without iron accumulation (25). Whether the classification of KRS as NBIA type 3 will turn out to be relevant remains speculative.

DAT scan showed markedly reduced uptake almost symmetrically in striatum in two patients, in line with the findings by Brüggemann et al. (11); however, an early more asymmetrical density reduction might be expected in cases with initial asymmetric motor symptoms, as reported for three of our patients. The indel mutation c.2473C>AA results in a frame-shift at position p.Leu825 in the protein and creates a premature stop codon, resulting in an open reading frame encoding a chimeric mutant protein. The mutant transcript was present in samples from two affected individuals (V-3 and V-9), and the wild-type transcript in two non-affected individuals (both children of V-5), excluding NMD of the mutant c.2473C>AA transcript. This is in contrast to the observations by Park et al. (12), who find the mutant c.3253delC (Table 2) level reduced and probably degraded by NMD in RNA from patient ‘human olfactory neurosphere-derived’ cells. The fact that NMD is observed for the c.3253delC mutation and not for the c.2473C>AA indel mutation suggests that different ATP13A2 mutations result in different pathogenic mechanisms and supports the observations of milder phenotypes reported in carriers (11) and in sporadic cases of ATP13A2.
with heterozygous mutations (Table 2). The fact that the mutation carriers in the Inuit family did not have mild KRS symptoms does not rule out that other ATP13A2 mutations can cause milder symptoms in the carrier state, as reported (11). A total of nine KRS families, including this family, are reported, and they carry 11 different mutations (Table 2). Only two cases of a ‘typical KRS phenotype’ have been reported in carriers (11, 16) (mutation c.3057delC and c.1108_1120del13, Table 2).

Milder KRS phenotypes have been reported in seven sporadic cases carrying single nucleotide variants (SNVs), representing missense mutations, and at least one, p.Ale746Thr, is suggested doubt-ful in several reports (17–20). The majority of these SNVs involve conserved or moderately con-served amino acids (Table 2), but the pathogenic mechanism is unknown.

In conclusion, we describe the highly variable and complex phenotype of six patients from an Inuit family, homozygous for a novel frameshift mutation in ATP13A2. The patient with the latest reported age at onset (29 years) had ataxia and axonal neuropathy, previously unrecognized in KRS, but only subtle parkinsonian features. Eight heterozygous carriers were all asymptomatic, and the consequence of the mutation cannot be explained by NMD of the mutant transcript.

Acknowledgements

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References


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