Ataxia Syndrome With Hearing Loss and Nephronophthisis Associated With a Novel Homozygous Variant in XPNPEP3

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Neurol Genet 2023;9:e200100. doi:10.1212/NXG.00000000000200100

Abstract

Objectives
Biallelic variants in XPNPEP3 are associated with a rare mitochondrial syndrome characterized by nephronophthisis leading to kidney failure, essential tremor, hearing loss, seizures, and intellectual disability. Only 2 publications on this condition are available. We report a man with a complex ataxia syndrome, hearing loss, and kidney failure associated with a new biallelic variant in XPNPEP3.

Methods
Clinical evaluation, neuroimaging studies, a kidney biopsy, and whole genome sequencing (WGS) were applied. Since the phenotype was compatible with a mitochondrial disease, a muscle biopsy with morphological and mitochondrial biochemical investigations was performed.

Results
Axial ataxia, cerebellar atrophy, hearing loss, myopathy, ptosis, supranuclear palsy, and kidney failure because of nephronophthisis were the prominent features in this case. WGS revealed the novel biallelic variant c.766C>T (p.Gln256*) in XPNPEP3. A muscle biopsy revealed COX negative fibers, a few ragged red fibers, and ultrastructural mitochondrial changes. Enzyme activity in respiratory chain complex IV was reduced in muscle and fibroblasts.

Discussion
This is the first report of a slowly progressive cerebellar ataxia associated with a novel biallelic variant in XPNPEP3. Abnormalities typical for mitochondrial disease and the slow progression of kidney disease are also striking. Our report expands the spectrum of XPNPEP3-related diseases.

Introduction
The protean symptoms and signs in mitochondrial disease include variable neurologic features. The X-prolyl aminopeptidase 3 (XPNPEP3) gene encodes a mitochondrial aminopeptidase involved in cleavage of matrix proteins. Biallelic pathogenic variants in XPNPEP3 are associated with nephronophthisis-like nephropathy 1 (OMIM # 613159). O'Toole et al. reported 2 families (5 patients) featuring nephronophthisis and variable neurologic signs, whereas isolated early-onset nephronophthisis was reported once. Associated symptoms include tremor, sensorineural hearing loss, seizures, intellectual disability (ID), cardiomyopathy, and pancreatic cysts. We report a man presenting with ataxia, hearing loss, myopathy, and chronic kidney failure associated with a novel homozygous truncating variant in XPNPEP3.
Methods

Details provided in eAppendix 1 (links.lww.com/NXG/A630).

Results

Case Presentation

A 53-year-old man from the Norrbotnian region in Sweden with a history of chronic kidney disease and gout presented with action and postural tremor, involuntary jerks, gait difficulties, falls, dysarthria, and loss of sensation in his feet. Onset was insidious and occurred during his late teens; this disorder was progressive and motivated the use of a walker a few months before his last visit. He was diagnosed with bilateral sensorineural hearing loss and stuttering speech at age 4 years; he reached his developmental milestones as expected and attended a regular school but had trouble acquiring motor skills (e.g., ride a bicycle and doing winter sports). His mother and deceased father have ancestry in the Finnish side of the Tornio valley, and none of them suffered from neurologic disease (Pedigree shown in eFigure 1, links.lww.com/NXG/A631). At age 23 years, the patient was evaluated for mitochondrial disease; however, a muscle biopsy and analyses of respiratory chain complexes were interpreted as normal then. EMG demonstrated mild myopathic abnormalities. Protein electrophoresis, cytology analysis, lactate, and protein levels in the CSF were normal. EEG demonstrated bilateral synchronous slow wave activity. Targeted analysis of common disease-causing variants in mitochondrial DNA (mtDNA) did not detect m.3243A>G, m.8344A>G, or m.8993T>G/C. Examination at age 53 years demonstrated predominant axial ataxia, dysmetria, dysarthria, postural and action tremor, slow vertical saccades, and ptosis were also found (Video 1 and eFigure 2, links.lww.com/NXG/A632). Examination with scale and rating of ataxia (SARA) yielded 17 points. In addition, the Romberg test was abnormal, pinprick sensation was reduced in both feet, and vibratory sensation was absent in the left lateral malleolus. During the examination, intermittent dystonic postures (feet and neck) and myoclonic jerks were also found. Muscle tonus was normal, but strength was reduced in both hands and legs (eAppendix 1, links.lww.com/NXG/A630), reflexes were symmetrical, and plantar response was flexion. Treatment with clonazepam reduced his myoclonic jerks. Neurography demonstrated a moderate axonal sensorimotor neuropathy and increased thresholds for temperature. Brain MRI demonstrated severe and progressive cerebellar atrophy and global cortical atrophy grade 1–2 more pronounced in the frontoparietal lobes (Figure 1, A and B). Extended laboratory work-up for ataxias, including for neurometabolic disorders, did not provide any diagnostic clues other than mild CK and neurofilament elevation in plasma. A recent echocardiography ruled out cardiomyopathy.

A psychometric evaluation demonstrated mild impairments, the patient obtained 76–90 index points on the working memory part (12th percentile) and 74–88 points on the perceptual function part (9th percentile) of WAIS-IV. The patient demonstrated perseveration, reduced concentration capacity and slower processing speed. Chronic kidney failure was diagnosed at age of 23 years. A kidney biopsy displayed features interpreted as glomerulonephritis (eAppendix 1, links.lww.com/NXG/A630). Estimated glomerular filtration rate was 40 mL/min/1.73 m² at age 23 years and slowly declined to 17 mL/min/1.73 m² at his latest visit and urea was 35.6 mmol/L (normal value: 3.5–8.2 mmol/L) (Table 1). Alport’s disease was considered at this point, but no variants in COL4A5 were identified. A re-evaluation of earlier kidney biopsy was pursued based on the genetic findings. Indeed, the biopsy demonstrated abnormalities compatible with nephronophthisis. Looming dialysis has raised kidney transplantation into consideration.

Genetic Analysis

WGS of DNA derived from blood detected a novel homozygous nonsense variant, c.766C>T, (p.Gln256*), in exon 4 of XPNPEP3 (NM_022098.4). His mother is heterozygous carrier of the variant. No DNA from the father was available for analysis. The variant is rare with a frequency of 24/251140

Figure 1 Neuroimaging of a Man With Ataxia Associated With a Homozygous XPNPEP3 Variant

(A) Midsagittal T1-weighted image shows severe cerebellar atrophy. (B) Axial T1-weighted image shows global atrophy more pronounced in the frontoparietal lobes.
Table 1 Laboratory Values for a 53-Year-Old Man With a Complex Neuro-renal Syndrome Associated With a Homozygous Variant in XPNPEP3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Normal reference value</th>
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<tr>
<td>GFR</td>
<td>17b</td>
<td>&gt;60 mL/min/1.73 m²</td>
</tr>
<tr>
<td>Sodium</td>
<td>141</td>
<td>137–145 mmol/L</td>
</tr>
<tr>
<td>Potassium</td>
<td>4.2</td>
<td>3.6–4.6 mmol/L</td>
</tr>
<tr>
<td>Urate</td>
<td>454</td>
<td>230–480 µmol/L</td>
</tr>
<tr>
<td>Ionic calcium</td>
<td>1.18</td>
<td>1.15–1.33 mmol/L</td>
</tr>
<tr>
<td>Phosphate</td>
<td>1.77b</td>
<td>0.75–1.40 mmol/L</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>130b</td>
<td>134–170 g/L</td>
</tr>
<tr>
<td>MCV</td>
<td>99b</td>
<td>82–98 fl</td>
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<tr>
<td>Platelet count</td>
<td></td>
<td>145–348 10⁹/L</td>
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<tr>
<td>White blood count</td>
<td>7.3</td>
<td>3.5–8.8 10⁹/L</td>
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<tr>
<td>Albumin</td>
<td>39</td>
<td>36–45 g/L</td>
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<tr>
<td>PTHb</td>
<td>32b</td>
<td>1.8–11 pmol/L</td>
</tr>
<tr>
<td>Urea</td>
<td>35.6b</td>
<td>3.5–8.2 mmol/L</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>3.67b</td>
<td>3.9–7.8 mmol/L</td>
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<tr>
<td>Triglycerides</td>
<td>2</td>
<td>0–2.6 mmol/L</td>
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<tr>
<td>HDL cholesterol</td>
<td>1.1</td>
<td>0.8–2.1 mmol/L</td>
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<tr>
<td>LDL cholesterol</td>
<td>1.7b</td>
<td>2.0–5.3 mmol/L</td>
</tr>
<tr>
<td>CK</td>
<td>13.5b</td>
<td>0–4.7 µkat/L</td>
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Abbreviations: GFR = glomerular filtration rate; MCV = mean corpuscular value; PTH = parathyroid hormone. 
* MCV has been between 95–99 fl during the last year. PTH level went up to 101 pmol/L a few months later. 
* Indicates abnormal value.

alleles in the general population and no homozygous individuals have been found, according to GnomAD (v2.1.1). The analysis detected also the heterozygous variant c.1997C>T, (p.Ala666Val), in PUM1 (NM_001020658.2) inherited from the healthy mother, which was interpreted as a variant of uncertain significance (eAppendix 1, links.lww.com/NXG/A633). WGS of DNA extracted from muscle did not detect any pathogenic variants in mtDNA.

**Biochemical and Morphological Analysis**

A skin biopsy and a second muscle biopsy were obtained at age 53 years. COX negative fibers and a few ragged red fibers (RRF) were found under light microscopy, whereas electron microscopy demonstrated paracrystalline “parking lot” inclusions and rounded electron dense structures within the mitochondria as well as abnormal cristae (Figure 2, A–D). In mitochondria isolated from muscle, ATP production using the complex IV dependent substrate combination TMPD+Ascorbate was reduced (eFigure 3A, links.lww.com/NXG/A633). Also, activity for the respiratory chain complex IV (CIV) was decreased (eFigure 3B). Respiratory chain enzymes in mitochondria isolated from fibroblasts showed decreased activity for CIV and an activity in the lower normal range for complex I (CI) (eFigure 3C). Coenzyme Q10 in the muscle specimen was 1.43 ng/unit CS (Normal value >1.18), treatment with coenzyme Q10 was started despite the normal value. Western blot analysis confirmed loss of XPNPEP3 protein in both cultured fibroblasts and muscle tissue from the patient (eFigure 4, A and B, links.lww.com/NXG/A634). In healthy controls, protein expression was much lower in skeletal muscle compared to cultured fibroblasts. This finding is somewhat in line with previous work. We applied the same antibody for XPNPEP3 reported in the Human Protein Atlas (Tissue expression of XPNPEP3 - Staining in skeletal muscle - The Human Protein Atlas, proteinatlas.org/). In this atlas no expression of XPNPEP3 is found; however, we found a low degree of expression in skeletal muscle (eFigure 4B).

**Discussion**

This is the first report of cerebellar ataxia and cerebellar atrophy, myopathy and long survival in association with a biallelic variant in XPNPEP3. In addition, morphological hallmarks for mitochondrial disease including ultrastructural abnormalities, COX negative fibers and RRF, are described for the first time in association with XPNPEP3 variants.

The combined findings of a homozygous nonsense variant, loss of protein expression in fibroblasts and skeletal muscle, clinical presentation and abnormalities in the muscle biopsy support pathogenicity for the c.766C>T variant in XPNPEP3. The crystal structure of other reported variants in XPNPEP3, c.931_934del (leading to frameshift and a stop codon at amino acid position 316) and c.1357G>T (leading to aberrant splicing, subsequent frameshift, and a stop codon at amino acid position 470), supports that both variants lead to structural collapse. The nonsense variant c.766C>T we report here leads to a stop codon and truncation of the protein at position 256 (p.Gln256*). The assumption that mRNAs, with such early stop codons, are degraded by nonsense mediated decay, is supported by loss of protein expression in fibroblasts and skeletal muscle from the patient. Normal findings in the muscle biopsy at age 23 years may suggest that abnormalities compatible with mitochondrial disease may appear after long disease duration. The protein expression of XPNPEP3 is absent in both skeletal muscle and fibroblasts in the patient and is low, but not absent, in skeletal muscle in healthy controls that could explain the muscle phenotype. The importance of XPNPEP3 for mitochondrial function is supported by the fact that deleting the orthologue gene icp55 in yeast leads to decreased oxygen consumption and ATP synthase complex assembly. Signs of mitochondrial defect have also been seen in other cases of XPNPEP3-associated disease. O’Toole et al. reported decreased CI activity in muscle of one patient and in fibroblasts from the other patient in a Turkish sibling pair harboring the homozygous c.931_934del variant. Full examination of the complexes of the respiratory chain has, however, not been carried out in these cases, e.g., only CI was analyzed in fibroblasts.
O’Toole et al. reported variable neurologic features in association with nephronophthisis. In one family from northern Finland with 3 affected siblings, essential tremor (ET) was found in all, whereas 2 siblings had sensorineural hearing loss. In addition, a Turkish family with 2 affected siblings suffered from severe intellectual disability (ID), seizures, cardiomyopathy, and pancreatic cysts. Neuroimaging data, which demonstrated arachnoid cysts, was provided only for one of the affected Finnish siblings. Myoclonus, as in our patient, can be a manifestation seen in uremia. Striking findings in this case are the slow progression of both neurologic and renal features, late-onset complex movement disorder, cerebellar atrophy, myopathy, clear morphological abnormalities in muscle associated with mitochondrial disease, and reduced activity in the CIV in muscle and fibroblasts. The rate of kidney failure was also slower in this case compared with previously reported who had an early need for dialysis. Taken together, our findings expand the spectrum of disorders associated with variants in XPNPEP3 (eTable 1, links.lww.com/NXG/A635). Early onset, intellectual disability, and cardiomyopathy were additional features in 2 Turkish siblings harboring a frame shift variant in XPNPEP3 and deficits in CL. However, genotype-phenotype correlations are not possible to establish because of scarcity of cases. In addition, the neuropathology of this disease remains to be studied. The pattern of manifestations is striking, considering the ubiquitous pattern of expression for XPNPEP3, including the brain. The main differential diagnosis includes Kearns-Sayre syndrome and epilepsy, and progressive myoclonic 4, with or without renal failure (OMIM # 254900).

Other ataxia cases associated with XPNPEP3 are required to delineate an association with this gene. Because ET, reported in previous cases, has a cerebellar generator, we suggest that long disease duration leading to ataxia as in our case may be part of the natural history of neurologic features associated with XPNPEP3.

Acknowledgment
The Promobilia Foundation, Region Stockholm.

Study Funding
The authors report no targeted funding.

Disclosure
The authors report no relevant disclosures. Go to Neurology.org/NG for full disclosures.

Publication History
Received by Neurology: Genetics June 7, 2023. Accepted in final form August 4, 2023. Submitted and externally peer reviewed. The handling editor was Editor Stefan M. Pulst, MD, Dr med, FAAN.

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<th>Location</th>
<th>Contribution</th>
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<tbody>
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Appendix (continued)

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<th>Location</th>
<th>Contribution</th>
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<td>Major role in the acquisition of data and analysis or interpretation of data</td>
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References

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DOI 10.1212/NXG.0000000000200100

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