CLINICAL/SCIENTIFIC NOTE OPEN ACCESS

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

Ilan Ben-Shabat, MD, Malin Kvarnung, MD, PhD, Wolfgang Sperker, MD, Helene Bruhn, MSc, Anna Wredenberg, MD, PhD, Rolf Wibom, PhD, Inger Nennesmo, MD, PhD, Martin Engvall, MD, PhD, and Martin Paucar, MD, PhD

Correspondence
Dr. Paucar
martin.paucar-arce@sll.se

Neurol Genet 2023;9:e200100. doi:10.1212/NXG.0000000000200100

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.

MORE ONLINE

Video

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.[1] Biallelic pathogenic variants in *XPNPEP3* are associated with nephronophthisis-like nephropathy 1 (OMIM # 613159). O'Toole et al.[2] reported 2 families (5 patients) featuring nephronophthisis and variable neurologic signs, whereas isolated early-onset nephronophthisis was reported once.[3] Associated symptoms include tremor, sensorineural hearing loss, seizures, intellectual disability (ID), cardiomyopathy, and pancreatic cysts.[2] We report a man presenting with ataxia, hearing loss, myopathy, and chronic kidney failure associated with a novel homozygous truncating variant in *XPNPEP3*.

From the Departments of Neurology (I.B.-S.) and Internal Medicine (W.S.), Sunderby Hospital, Luleå; Umeå University (I.B.-S.); Department of Clinical Genetics (M.K.), Centre for Inherited Metabolic Diseases (H.B., A.W., R.W., M.E.), Karolinska University Hospital, Stockholm; Departments of Medical Biochemistry and Biophysics (A.W.), Oncology and Pathology (I.N.), Molecular Medicine and Surgery (M.E.), and Neurology (M.P.), Karolinska Institutet, Stockholm, Sweden.

Go to Neurology.org/NG for full disclosures. Funding information is provided at the end of the article.

The Article Processing Charge was funded by the authors.

Methods

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

Results

Case Presentation

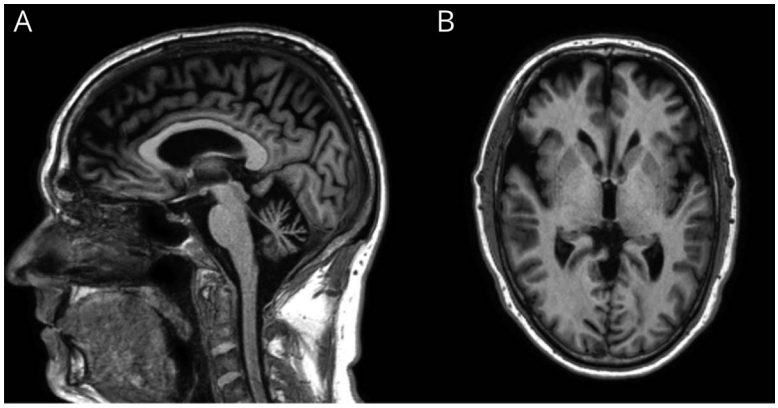
A 53-year-old man from the Norrbotnian region in Sweden with a history of chronic kidney disease and gout presented with action and postural tremor, involuntary jerks, gait difficulties, falls, dysarthria, and loss of sensation in his feet. Onset was insidious and occurred during his late teens; this 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 m^2 at age 23 years and slowly declined to 17 mL/min/1.73 m^2 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*

Parameter	Value	Normal reference value
GFR	17[b]	>60 mL/min/1.73 m^2
Sodium	141	137–145 mmol/L
Potassium	4.2	3.6–4.6 mmol/L
Urate	454	230–480 μmol/L
Ionic calcium	1.18	1.15–1.33 mmol/L
Phosphate	1.77[b]	0.75–1.40 mmol/L
Hemoglobin	130[b]	134–170 g/L
MCV[a]	99[b]	82-98 fL
Platelet count	208	145-348 10^9/L
White blood cell count	7.3	3.5–8.8 10^9/L
Albumin	39	36–45 g/L
PTH[b]	32[b]	1.8–11 pmol/L
Urea	35.6[b]	3.5–8.2 mmol/L
Cholesterol	3.67[b]	3.9–7.8 mmol/L
Triglycerides	2	0–2.6 mmol/L
HDL cholesterol	1.1	0.8–2.1 mmol/L
LDL cholesterol	1.7[b]	2.0–5.3 mmol/L
CK	13.5[b]	0–4.7 μkat/L

Abbreviations: GFR = glomerular filtration rate; MCV = mean corpuscular value; PTH = parathyroid hormone.
[a] MCV has been between 95-99 fL during the last year. PTH level went up to 101 pmol/L a few months later.
[b] 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/A630). WGS of DNA extracted from muscle did not detect any pathogenic variants in mtDNA.

Biochemical and Morphological Analysis

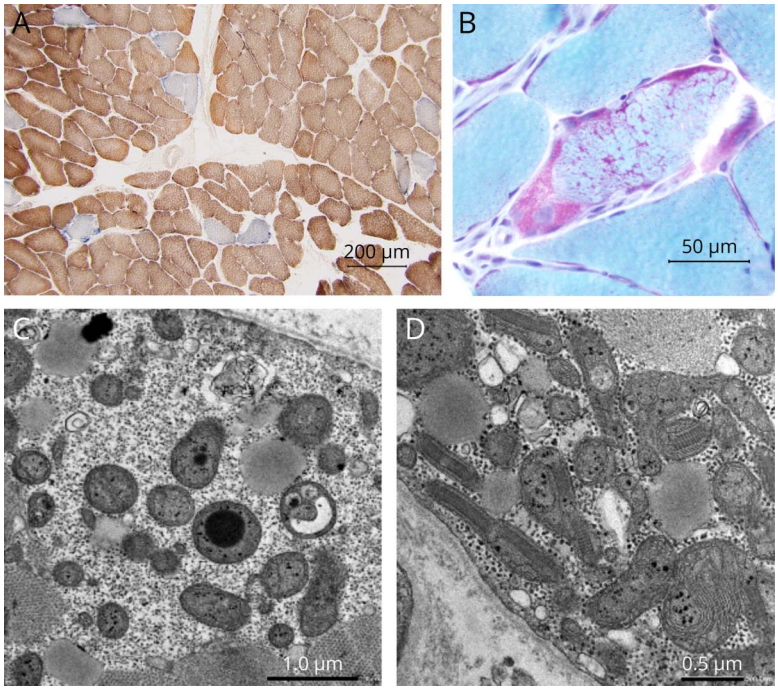
A skin biopsy and a second muscle biopsy were obtained at age 53 years. COX negative fibers and a few ragged red fibers (RRF) were found under light microscopy, whereas electron microscopy demonstrated paracrystalline "parking lot" inclusions and rounded electron dense structures within the mitochondria 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.[4] 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.[5] 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.[2]

Figure 2 Muscle Biopsy From a Man With Ataxia Associated With a Homozygous *XPNPEP3* Variant



(A) COX-SDH reaction showing fibers (blue) lacking COX-activity, bar 200 μm. (B) A ragged red fiber is shown, Gomori trichrome, bar 50 μm. (C) Electron microscopy showing mitochondria with abnormal cristae. In one of these mitochondria, there is a large dense rounded inclusion, bar 1.0 μm. (D) Mitochondria containing parking lot inclusions, bar 0.5 μm.

O'Toole et al. reported variable neurologic features in association with nephronophthisis.[2] 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.[2] 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.[2,3] 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 CI.[2] 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 brain6. The main differential diagnosis includes Kearns-Sayre syndrome and epilepsy, and progressive myoclonic 4, with or without renal failure (OMIM # 254900).[7,8]

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.

Appendix Authors

Name	Location	Contribution
Ilan Ben-Shabat, MD	Department of Neurology, Sunderby Hospital, Luleå, Sweden and Umeå University, Sweden	Major role in the acquisition of data and analysis or interpretation of data

Appendix *(continued)*

Name	Location	Contribution
Malin Kvarnung, MD, PhD	Department of Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden	Analysis or interpretation of data
Wolfgang Sperker, MD	Department of Internal Medicine, Sunderby Hospital, Luleå, Sweden	Analysis or interpretation of data
Helene Bruhn, MSc	Centre for Inherited Metabolic Diseases, Karolinska University, Stockholm, Sweden	Analysis or interpretation of data
Anna Wredenberg, MD, PhD	Centre for Inherited Metabolic Diseases, Karolinska University, Stockholm, Sweden and Department Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden	Analysis or interpretation of data
Rolf Wibom, PhD	Centre for Inherited Metabolic Diseases, Karolinska University, Stockholm, Sweden	Major role in the acquisition of data
Inger Nennesmo, MD, PhD	Department Oncology and Pathology, Karolinska Institutet, Stockholm, Sweden	Major role in the acquisition of data and analysis or interpretation of data
Martin Engvall, MD, PhD	Centre for Inherited Metabolic Diseases, Karolinska University, Stockholm, Sweden and Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden	Major role in the acquisition of data and analysis or interpretation of data
Martin Paucar, MD, PhD	Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden and Department of Neurology, Karolinska University Hospital, Stockholm, Sweden	Major role in the acquisition of data; study concept or design; and analysis or interpretation of data

References

1. Wachoski-Dark E, Zhao T, Khan A, Shutt TE, Greenway SC. Mitochondrial protein homeostasis and cardiomyopathy. *Int J Mol Sci.* 2022;23(6):3353. doi:10.3390/ijms23063353
2. O'Toole JF, Liu Y, Davis EE, et al. Individuals with mutations in XPNPEP3, which encodes a mitochondrial protein, develop a nephronophthisis-like nephropathy. *J Clin Invest.* 2010;120(3):791-802. doi:10.1172/jci40076
3. Alizadeh R, Jamshidi S, Keramatipour M, et al. Whole exome sequencing reveals a XPNPEP3 novel mutation causing nephronophthisis in a pediatric patient. *Iran Biomed J.* 2020;24(6):405-408. doi:10.29252/ibj.24.6.400
4. Singh R, Jamdar SN, Goyal VD, Kumar A, Ghosh B, Makde RD. Structure of the human aminopeptidase XPNPEP3 and comparison of its in vitro activity with Icp55 orthologs: insights into diverse cellular processes. *J Biol Chem.* 2017;292(24):10035-10047. doi:10.1074/jbc.m117.783357
5. Stames EM, O'Toole JF. Mitochondrial aminopeptidase deletion increases chronological lifespan and oxidative stress resistance while decreasing respiratory metabolism in S. cerevisiae. *PLoS One.* 2013;8(10):e77234. doi:10.1371/journal.pone.0077234
6. Ersahin C, Szpaderska AM, Orawski AT, Simmons WH. Aminopeptidase P isozyme expression in human tissues and peripheral blood mononuclear cell fractions. *Arch Biochem Biophys.* 2005;435(2):303-310. doi:10.1016/j.abb.2004.12.023
7. Badhwar A, Berkovic SF, Dowling JP, et al. Action myoclonus-renal failure syndrome: characterization of a unique cerebro-renal disorder. *Brain.* 2004;127(Pt 10):2173-2182. doi:10.1093/brain/awh263
8. Govers LP, Toka HR, Hariri A, Walsh SB, Bockenhauer D. Mitochondrial DNA mutations in renal disease: an overview. *Pediatr Nephrol.* 2021;36(1):9-17. doi:10.1007/s00467-019-04404-6