Supplementary document

**Complex ataxia syndrome with hearing loss and nephronophthisis associated with a novel homozygous variant in XPNPEP3**

1. **Additional clinical data**

This patient was evaluated by nephrologists at two different centers. A kidney biopsy at age 23 years demonstrated glomerulosclerosis, focal interstitial fibrosis and atrophic tubule. Immunofluorescent microscopy was normal, taken together membranous glomerulonephritis was suspected. This biopsy specimen was re-evaluated and found to display typical features for nephronophthisis. The patient is treated for chronic kidney failure (stadium 4). Thus, a slow decline in GFR has been documented over a long time, during the last year his GFR has been between 16-18 ml/min/1.7 (Normal value >60). As expected for somebody with chronic kidney disease, urea, potassium, parathyroid hormone, and phosphate levels (Table 1) were elevated motivating treatment with natrium bicarbonate, polystyrene sulfonate, cinacalcet and sevelamer. Subsequently his calcium levels were low. Hypertension was treated with furosemide, propranolol, and amlodipine. He was also treated with atorvastatin, and allopurinol. The patient has a mild normocytic anemia but does not required treatment with erythropoietin yet. There is no evidence of proteinuria either. We refrained from a second kidney biopsy due to the higher risks for this procedure in severe kidney failure. Dialysis is planned to start in the coming months, kidney transplantation has been proposed.

Assessment of muscle strength Medical Research Council (MRC) was normal in the upper arm abductors, and elbow flexors (MRC 5), but reduced (MRC 4) in in the wrist extensors, hip flexors, knee extensors and foot dorsal flexors. For his neurological symptoms he is treated with clonazepam, and coenzyme Q10.

2. **Methods**

**Genetic analyses**

Genomic DNA (gDNA) was extracted from blood and a muscle specimen according to standard procedures. Whole genome sequencing (WGS) of genomic DNA from blood and muscle was performed.
to a sequencing depth of 35x mean coverage using a NovaSeq 6000 sequencing instrument (Illumina) after library preparation with NxSeq AmpFREE Low DNA Library Kit (Lucigen). This was followed by in-house bioinformatics analysis, using the mutation identification pipeline (MIP) as earlier described (e-1). All known disease genes associated with mitochondrial, metabolic or neuromuscular disease were analyzed, using precompiled gene panels that are available at https://www.karolinska.se/forvardgivare/karolinska-universitetslaboratoriet/genomic-medicine-center-karolinska/gmck-rare-diseases/. Supplementary analysis of data from genes associated with the human phenotype ontology (HPO) terms ataxia (HP:0001251), hearing impairment (HP:0000365), polyneuropathy (HP:0001271), myoclonic spasms (HP:0003769), cerebellar atrophy (HP:0001272) and paraparesis (HP:0002385) was also performed. Mitochondrial DNA (mtDNA) extracted from a muscle biopsy was analyzed with WGS. Neither intronic nor exonic pathological nucleotide expansions in genes associated with neurological diseases were detected (Expansion Hunter).

The $PUM1$ variant found in this case and in his mother is rare with a frequency of 20/282664 alleles in GnomAD. This variant was found in another individual, with intellectual disability but no signs of ataxia or kidney disease, in our database containing WGS data from 7678 individuals. Different tools yielded contradicting predictions, SIFT predicted it as deleterious (score = 0.05), HumDiv as possibly damaging (score = 0.56) whereas HumVar (score = 0.18) and Polyphen predicted it as benign. Taken together, this $PUM1$ change is judged to be a variant of unclear significance (VUS).

**Biochemical and morphological analyses**

A biopsy was obtained from the right tibialis anterior muscle and handled according to standard protocols for light and electronic microscopy. Mitochondria were isolated from muscle and mitochondrial ATP production rate (MAPR) and respiratory chain enzyme activities were determined as previously described (e-2). Respiratory chain enzyme activities in mitochondria isolated from fibroblasts were determined using the same methods.

**Western blotting**
Isolated mitochondria from muscle tissue and cultured fibroblasts were mixed with NuPAGE™ sample buffer and reducing reagent according to the manufacturer’s instructions (ThermoFisher Scientific) and an equivalent of 5 µg protein was loaded on a 4-12% NuPAGE™ Bis-Tris 1.0 mm gel. Gel was run for 40 min at 200 V in NuPAGE™ running buffer and transferred with an iBlot™ system (ThermoFisher Scientific) onto PVDF membrane with pore size 0.45 µM, which was blocked in blocking buffer (5% milk in Tris buffer saline with 1% Tween 20). Immunoblotting was performed using standard techniques with primary antibodies XPNPEP3 (PA5-51402, Invitrogen, 1:1000, over night) and VDAC (ab15895, Abcam, 1:1000, 1 hour) and secondary antibody (Rabbit IgG HRP Linked Whole Ab, NA934, Cytiva™, 1:5000, 1 hour). Development was performed with Clarity Western ECL Substrate (Bio-Rad Laboratories).

**e-References**
