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Mitochondriopathy Manifesting as Inherited Tubulointerstitial Nephropathy Without Symptomatic Other Organ Involvement

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INTRODUCTION

Mitochondria are of ancient prokaryotic origin yet are ubiquitously present in eukaryotic cells. They have their own circular, double-stranded DNA (mtDNA), which, in humans, is maternally inherited. During cytolysis, mitochondria are randomly distributed among the daughter cells. Moreover, each mitochondrion replicates independently of the others. If a mutation occurs in 1 mitochondrion, the more that organelle divides and fuses, the more of the newly generated mitochondria will carry the mutation. The presence of coexisting mtDNAs (i.e., wild-type and mutated) is referred to as heteroplasmy. Often, if a mutation is pathogenic, the severity of a mitochondrial disease depends on the percentage of heteroplasmy, with a status of "mutant homoplasmy" (100% mutated mtDNA) usually resulting in the most severe clinical phenotype.

Mitochondrial dysfunction has been previously described in cases of chronic kidney disease (CKD).[1] Impaired adenosine triphosphate (ATP) production, reactive oxygen species generation, and inflammation induction are some possible mechanisms whereby organ damage is mediated. Individuals affected by primary systemic mitochondrial disease may develop kidney injury and eventually CKD. Importantly, mitochondrial disease associated with organ-limited dysfunction has also been described, but, at this time, remains exceedingly rare. In the kidney, Connor *et al*. recently reported 2 examples of mitochondrially inherited tubulointerstitial kidney disease in patients with no apparent other organ involvement.[2]

In the current report, we describe a family affected by a mitochondrially inherited tubulointerstitial kidney disease. Despite a homoplasmic mitochondrial alteration detected in the blood, the only signs and symptoms present were related to the kidney.

CASE PRESENTATION

The patient is a 12-year-old boy with a significant family history of CKD (Figure 1). Both the mother and maternal uncle have end-stage renal disease of unknown etiology and have been on dialysis since their 30s. Of note, the patient has a 10-year-old half-brother (same mother) with a clinical diagnosis of Bartter syndrome. This diagnosis was made based on the detection of hypokalemia and metabolic alkalosis without performing genetic characterization. This half-sibling had a normal glomerular filtration rate (GFR) during the last 5 years.

The school nurse noticed that our proband had a short stature ($<5^{th}$ percentile for age and sex), and further laboratory workup was ordered. Serum creatinine was elevated at 1.9 mg/dl and blood urea nitrogen was 30 mg/dl. The patient had CKD stage 4 (GFR 27 ml/min per 1.73 m^2 body surface area based on revised Schwartz equation) along with comorbidities of CKD: anemia, secondary hyperparathyroidism, and vitamin

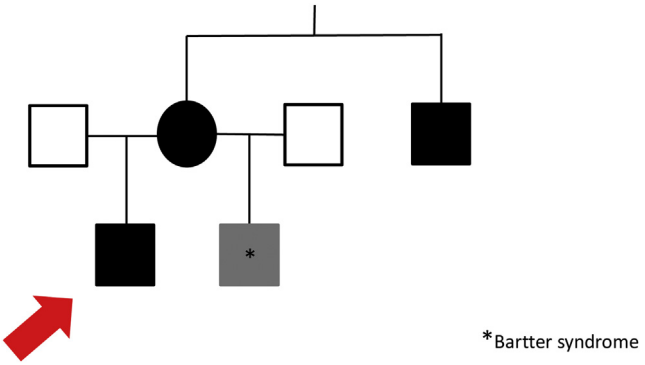

Figure 1. Patient's family. Red arrow indicates our patient (proband); black symbols indicate the relatives affected; gray symbol represents the half-brother diagnosed with Bartter syndrome. Circle indicates female; square indicates male.

D deficiency. Renal ultrasound showed bilateral echogenic small kidneys ($<5^{th}$ percentile for age and sex). Table 1 shows the relevant laboratory results of our proband at the time of presentation (Table 1). No other clinical symptoms or signs were present, and blood pressure was normal. A diagnostic kidney biopsy was then performed.

Light microscopy showed mild, nonspecific, chronic tubulointerstitial nephropathy. Occasional distal tubular cells exhibited swollen, eosinophilic, and granular cytoplasm (Figure 2a). Immunofluorescence was negative. Electron microscopy showed severely enlarged tubular epithelial cells filled with dysmorphic mega-mitochondria characterized by circularly arranged cristae and electron-dense, parallel linear inclusions (Figure 2b). Given this striking histopathologic finding, genetic testing for identifying a mitochondrial alteration was performed.

Next-generation sequencing was performed on frozen tissue. DNA was extracted as per standard clinical protocols by the Mayo Clinic Genomics Laboratory. The mitochondrial genome was amplified from patient samples by long-range polymerase chain reaction, and next-generation sequencing was performed on the PCR products using a TruSeq Nano library preparation sequenced on an Illumina MiSeq (primary) and an Ion Plus Fragment library preparation sequenced on an Ion Torrent Personal Genome Machine (confirmatory). Molecular testing showed the presence of a single missense alteration in position 616 (m.616T>C) of the mitochondrially encoded transfer RNA phenylalanine (*MTTF*) gene. This alteration was homoplasmic in the renal tissue. This result, combined with the pattern of inheritance and the renal biopsy findings for this patient, was strongly suggestive of a diagnosis of mitochondrially inherited tubulointerstitial kidney disease.

Table 1. Relevant laboratory results of our proband at presentation

Blood	
Creatinine	1.9 mg/dl (0.8–1.2)
Blood urea nitrogen	30 mg/dl (7–20)
Na	141 mmol/l (132–143)
K	4 mmol/l (3.6–5.1)
CO_2	22 mmol/l (21–33)
Hb	9.6 g/dl (11.6–15.9)
25-OH-Vit D	16.2 ng/ml (≥ 30.0)
Parathyroid hormone	176.1 pg/ml (12.0–88.0)
Urine	
Proteinuria	Negative <150 mg
Red blood cells	0–2 (0–2)

With the same methodology, we then analyzed the blood samples (leukocyte mtDNA) of the mother and maternal uncle and found the same mitochondrial alteration in homoplasmy. In addition, the 10-year-old half-brother with ostensible Bartter syndrome underwent biopsy. Light microscopy showed minimal cortical scarring; electron microscopy confirmed the same features of mitochondrial cytopathy seen in the proband (Figure 3), more prominent in distal tubules, with the same homoplasmic alteration.

Notably, the pattern of inheritance seen in this family could also be compatible with an autosomal dominant tubulointerstitial kidney disease (ADTKD). Thus, in the 2 half-siblings, we performed genotyping of the 2 most common genes associated with ADTKDs, *UMOD* and *MUC1*,[3] to exclude a mutation. For the analysis of these 2 genes, whole blood was collected, and DNA was isolated by standard methodology. Genetic testing for *MUC1* mutations was performed by the Broad Institute (Cambridge, MA).[4] Genetic testing for *UMOD* mutations was performed by Charles University by candidate gene Sanger sequencing (Prague, Czech Republic).[5] Importantly, no mutations were detected in either genes, further supporting the diagnosis of a mitochondrially inherited kidney disease.

DISCUSSION

The current report analyzes a case of a 12-year-old boy affected by a mitochondrially inherited tubulointerstitial kidney disease. The homoplasmic missense alteration found in his condition is in position 616 (thymine to cytosine) of the mtDNA, located in the *MTTF* gene. Importantly, after this unexpected diagnosis, we were able to assess the other family members presenting with a similar symptomatology (early-onset CKD). Indeed, the mother and maternal uncle were affected by the same mitochondrial alteration detected in the blood, as well as the half-brother who had the same alteration in the kidney. All the subjects studied were homoplasmic for m.616T>C.

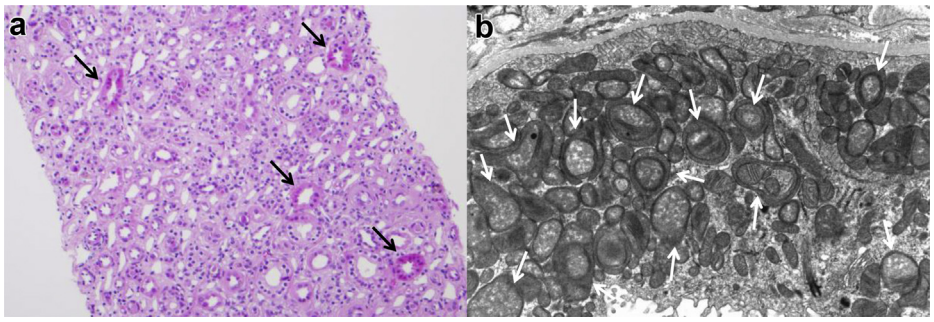

Figure 2. Kidney biopsy findings of patient (proband). (a) Light microscopy of this hematoxylin-and-eosin slide shows mild interstitial inflammation of this segment of the renal medulla. Scattered distal tubules show more eosinophilic and granular epithelial cells (arrows). These findings are nonspecific. (b) Electron microscopy shows numerous enlarged abnormal mitochondria, with circularly arranged cristae and electron-dense, parallel linear inclusions (arrows).

The *MTTF* gene, also called *TRNF*, is known for a few missense alterations (Table 2). The mitochondrial alteration detected in our case has been recently reported in a few families. Zsurka *et al.*[6] described a family that was affected mainly by severe familial, maternally inherited epilepsy. Their proband was a girl affected by severe epilepsy who died at the age of 17 years from complications related to status epilepticus; interestingly, she was also affected by CKD. Her maternal cousin was affected by severe epilepsy but died of kidney failure. Connor *et al.*[2] reported the m.616T>C in the *MTTF* gene in 3 families affected by inherited tubulointerstial renal disease. After identifying this alteration, the authors performed functional studies using patient fibroblasts and cybrids, and showed impaired cellular respiration compared to that in unaffected cells. The most intriguing finding was that none of the affected individuals had clear evidence of extrarenal disease; however, Burke *et al.*[7] previously reported that two different individuals belonging to 1 of the families also analyzed by Connor *et al.* had seizures. Importantly, all of these subjects had a homoplasmic mitochondrial alteration in all tissues tested (blood, skeletal muscle, and skin fibroblasts).

In our family, we also found a homoplasmic (i.e., 100% allele frequency) mutation in the blood of the affected subjects, yet with kidney-restricted manifestation. The reasons behind this apparent discrepancy could be multiple. The distal tubule of the nephron is very sensitive to oxygen deprivation. Indeed, ion pumps constantly work to maintain electrolyte balance requiring hydrolysis of ATP; dysfunctional mitochondria would compromise this vital energy supply. However, the same explanation could potentially apply to other tissue organs with many ATP-dependent functions, such as muscle and brain. Another explanation could be that the mitochondrial alteration may be present systemically but the percentage of heteroplasmy differs among organs, reflecting the kidney-limited manifestation. Overall, we favor that other pathways, systems, and microenvironmental conditions must play a role in the specificity of the final dysfunctional outcome, and further studies are warranted to investigate these potential modulators.

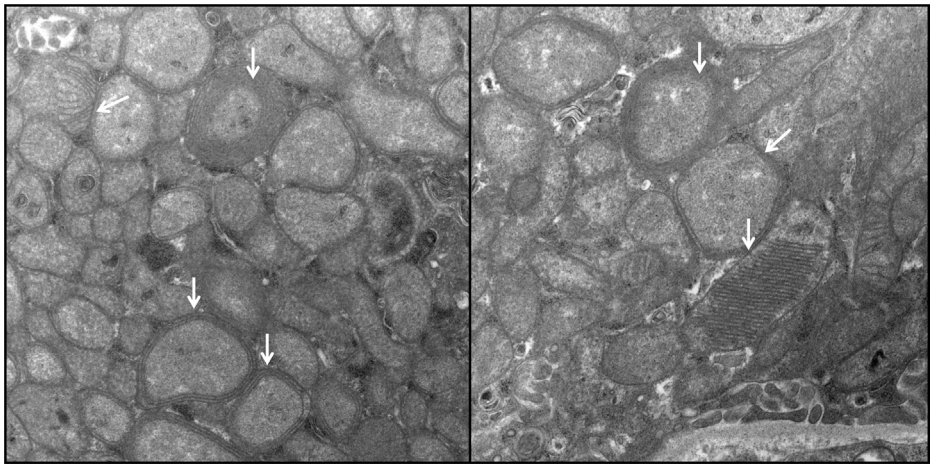

Figure 3. Kidney biopsy findings of patient's half-brother. Electron microscopy shows numerous enlarged dysmorphic mitochondria, with circularly arranged cristae and electron-dense, parallel linear inclusions (arrows).

Table 2. Allelic variants of the *MTTF* gene[a]

Phenotype	*MTTF* mutation
MELAS syndrome	583G-A
MERRF syndrome	611C-A
Late-onset myopathy	622G-A
Epilepsy	616T-C
Epilepsy	616T-G
Encephalopathy	586G-A
Tubulointerstitial nephropathy	608A-G

MELAS, mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes; MERRF, myoclonic epilepsy with ragged red fibers.
[a]Source: ClinVar.

Table 3. Teaching points

Tubulointerstitial diseases have multiple causes, including drug, infectious, immune-mediated, and genetic causes.
When mitochondrial dysmorphism is present, electron microscopy is the technique that allows for the identification of such abnormality.
In cases of familial or early-onset tubulointerstial diseases, examining the mitochondria is warranted.
Specific mitochondrial DNA mutations have been described causing mitochondrially inherited tubulointerstitial kidney disease, including the m.616T>C in the *MTTF* gene.
Recognizing a mitochondrially inherited tubulointerstitial kidney disease is important for diagnosis, prognosis, and treatment.
Kidney transplantation may be a possible therapeutic option in a mitochondrially inherited tubulointerstitial kidney disease.

Our patient's half-sibling carried a diagnosis of Bartter syndrome, a rare condition characterized by impairment of the electrolyte transporters in the thick ascending limb of the loop of Henle. Consequences of this dysfunction include electrolytes loss, volume depletion with impaired ability to concentrate urine, metabolic alkalosis, and secondary hyperaldosteronism. Bartter syndrome can present in childhood, and its severity varies depending upon the type of genetic mutation, occasionally leading to CKD; histologically it is characterized by hyperplasia of the juxtaglomerular apparatus. Our patient's unusual biopsy findings and family history prompted us to question the half-brother's initial diagnosis and to perform a kidney biopsy. Notably, in the latter, histological examination did not reveal hyperplasia of the juxtaglomerular apparatus. Also, the renal mitochondriopathy was more prominent in the distal tubules potentially explaining a Bartter-like phenotype.

Finally, the pattern of inheritance seen in this family along with the light microscopy findings could raise the question of a possible diagnosis of ADTKD. We performed genetic analysis to look for pathological mutations in *UMOD* and *MUC1*, the 2 most common genes involved in cases of ADTKD. Importantly, no mutations were identified in either genes, further supporting the diagnosis of a mitochondrial kidney disease.

In summary, we were able to confirm that this family was affected by a mitochondrially inherited tubulointerstitial kidney disease. This surprising diagnosis could potentially change the management of these individuals and result in a better treatment and prognosis, including strictly monitoring the half-brother's GFR. For a mitochondrially inherited tubulointerstitial kidney disease, in contrast to other systemic mitochondriopathies, kidney transplantation could be an early therapeutic option. Connor *et al.* reported long-term disease-free survival (>20 years) after kidney transplantation in subjects affected by tubulointerstitial kidney disease due to a different organ-limited homoplasmic mitochondrial alteration.[2] A kidney transplant could change the long-term quality of life and prognosis of our family. It is a consideration that this mitochondrial disease may not be organ limited; a relatively low heteroplasmic alteration status in other, non-tested organ systems may be currently silent and may become overt over time. In the current family, based on previous reports, the kidney dysfunction could be associated with a reduced threshold for epileptic seizures. However, this evolution is not certain, and should not preclude consideration of a kidney transplant.

CONCLUSION

In conclusion, this is a case of a family affected by a mitochondrially inherited tubulointerstitial kidney disease due to m.616T>C in the *MTTF* gene. Organ-restricted mitochondrial diseases should be kept in mind in the differential diagnosis of single-organ dysfunction, as they can mimic other diseases. Our case supports and reinforces the possibility of a single-organ–limited mitochondrial disease, regardless of the systemic mitochondrial DNA alteration status, potentially radically changing the management and outcome of these patients. Careful analysis of mitochondria by electron microscopy should be performed in patients with tubulointerstitial nephropathy and a family history of kidney failure. Table 3 provides teaching points concisely summarizing our important findings.

DISCLOSURE

All the authors declared no competing interests.

PATIENT CONSENT

The authors declared that they have obtained consent from the patients discussed in the report.

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