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Renal Phenotype in Mitochondrial Diseases: A Multicenter Study

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Keywords
Mitochondrial disease · Acute kidney injury · Mitochondrial DNA · Renal manifestations

Abstract
Aims: This study aimed to investigate associations between renal and extrarenal manifestations of mitochondrial diseases and their natural history as well as predictors of renal disease severity and overall disease outcome. The secondary aim was to generate a protocol of presymptomatic assessment and monitoring of renal function in patients with a defined mitochondrial disease. ***Methods:*** A multicenter, retrospective cohort study was performed by the Mitochondrial Clinical and Research Network (MCRN). Patients of any age with renal manifestations associated with a genetically verified mitochondrial disease were included from 8 expert European centers specializing in mitochondrial diseases: Gothenburg, Oulu, Copenhagen, Bergen, Helsinki, Stockholm, Rotterdam, and Barcelona. ***Results:*** Of the 36 patients included, two-thirds had mitochondrial DNA-associated disease. Renal manifestations were the first sign of mitochondrial disease in 19%, and renal involvement was first identified by laboratory tests in 57% of patients. Acute kidney injury occurred in 19% of patients and was the first sign of renal disease in the majority of these. The most common re-

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nal manifestation was chronic kidney disease (75% with stage 2 or greater), followed by tubulopathy (44.4%), the latter seen mostly among patients with single large-scale mitochondrial DNA deletions. Acute kidney injury and tubulopathy correlated with worse survival outcome. The most common findings on renal imaging were increased echogenicity and renal dysplasia/hypoplasia. Renal histology revealed focal segmental glomerulosclerosis, nephrocalcinosis, and nephronophthisis. ***Conclusion:*** Acute kidney injury is a distinct renal phenotype in patients with mitochondrial disease. Our results highlight the importance to recognize renal disease as a sign of an underlying mitochondrial disease. Acute kidney injury and tubulopathy are 2 distinct indicators of poor survival in patients with mitochondrial diseases.

Introduction

Mitochondrial diseases are a heterogeneous group of disorders caused by defects in the mitochondrial oxidative phosphorylation system. Multiple organs can be affected, especially those with high energy requirements, such as the central nervous system, skeletal muscle, heart, kidneys, and liver [1]. Renal dysfunction may be the presenting feature of mitochondrial diseases, but is most commonly seen after the onset of neurological manifestations, often as part of a multisystem phenotype [2]. The reported prevalence of renal involvement in mitochondrial diseases varies from 25 to 50% [3–5], and the underlying genetic etiology seems to play a role both in the varying prevalence and type of renal manifestations [3].

Reported renal manifestations of mitochondrial diseases include tubular dysfunction, interstitial nephritis, nephrotic syndrome, cystic disease, and end-stage renal failure [2, 3, 6, 7]. Proximal tubulopathy is the commonest phenotype in childhood-onset mitochondrial disease and can be associated with both nuclear DNA (nDNA) variants and large-scale mitochondrial DNA (mtDNA) deletions [6, 8]. Apparently, the high energy requirements and the inability to synthesize adenosine triphosphate anaerobically from glycolysis make proximal tubular cells extremely vulnerable to energy crisis secondary to mitochondrial dysfunction [9].

Severe tubular dysfunction, known as de Toni-Debré-Fanconi syndrome, occurs in patients with mtDNA deletion syndromes such as Kearns-Sayre and Pearson syndrome [6]. This syndrome is characterized by renal tubular acidosis, hyperphosphaturia, glycosuria, and generalized aminoaciduria with low-molecular-weight proteinuria [3, 6]. mtDNA point mutations such as m.3243A>G that cause mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes, and maternally inherited diabetes and deafness have been mostly associated with phenotypes such as tubulointerstitial nephritis, focal segmental glomerulosclerosis, and progressive renal failure [8, 10]. The renal phenotype of nDNA-associated mitochondrial disease is more diverse [8].

As mitochondrial diseases are often multisystemic and associated with severe neurological phenotypes, renal manifestations can go undetected until the renal symptoms are severe, or the patient has developed end-stage renal failure. Recognizing an underlying mitochondrial disease when renal symptoms occur first or alone can be equally difficult. Currently, there are few cohort studies available to inform our practice [3–5], the majority being clinical case reports and reviews. Therefore, we undertook this multinational cohort study to investigate associations between renal and extrarenal onset of mitochondrial disease, the natural history of renal manifestations, and predictors of renal disease severity and overall disease outcome. On the basis of our results, we propose a protocol for the assessment and monitoring of renal function in presymptomatic patients with known mitochondrial disease.

Methods

Patients

This multicenter, retrospective cohort study supported by the Mitochondrial Clinical and Research Network (MCRN) included patients from 8 expert European centers specializing in mitochondrial diseases: Gothenburg, Oulu, Copenhagen, Bergen, Helsinki, Stockholm, Rotterdam, and Barcelona. Patients of any age with a genetically defined mitochondrial disease with renal manifestations were included.

Data Collection

Data were collected using a standardized case report form (available upon request). Collected data included results from genetic testing, clinical, biochemical, morphological, and imaging assessments, comorbidities, and treatment used. As this was a retrospective study, a cutoff for missing data of 10% or less was implemented to ensure quality of statistical inferences. Assessments for 5 patients have been included in other publications, as detailed in Tables 1 and 2.

Clinical and Laboratory Variables

Occurrence and severity of renal disease was evaluated by estimating the amount of proteinuria and/or hematuria and by measuring the glomerular filtration rate (GFR) either directly by EDTA-clearance (in mL/min/1.73 m^2) or by calculating the estimat-

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Table 1. Age at disease onset, renal phenotype, mitochondrial extrarenal manifestations, and genetics ($n = 36$) [21–24]

ID	Gender	Age at mito onset, years	Age at kidney onset, years	First renal manifestations	AKI	Tubulopathy	CKD stage 2 or greater	Nephrotic syndrome	Nephritic syndrome	Hypertension	CNS (encephalopathy, epilepsy)	PNS (peripheral neuropathy)	Muscle weakness or muscle hypotonia	Exercise intolerance	Ocular manifestations	Cardiac manifestations[§]	Diabetes mellitus	Abnormal growth	Mitochondrial phenotype	Genetics
1	M	6	6	Hypertension			●			●		●							Nephronophthisis-like nephropathy	*XPNPEP3*
2	F	6	6	Fanconi syndrome		●	●			●	●		●		●	●	●	●	KSS	*LSMD*
3	M	28	28	Weight loss, polyuria			●	●			●								MELAS	*MTTL1*
4	M	22	22	Increased creatinine			●						●	●				●	MELAS	*MTTL1*
5	F	0.1	0.1	Increased amino acids and phosphate in urine	●	●					●								Complex III deficiency	*BCS1L*
6	M	0.1	0.1	Increased amino acids in urine	●	●					●								Complex III deficiency	*BCS1L*
7	M	0	0	Increased creatinine and kidney dysplasia	●					●		●	●		●	●		●	CoQ10 deficiency	*CoQ7*
8	M	0	4.9	Hypochloremic metabolic alkalosis with increased Na and K in urine	●	●		●			●				●			●	Alpers syndrome	*NARS2*
9	M	40.3	40.5	Increased creatinine	●	●	●				●	●					●		MELAS	*MTTL1*
10	M	1.6	13.3	Increased creatinine, hematuria, proteinuria	●						●		●					●	Complex I deficiency	*MTND1*
11	F	0.7	3.5	Fanconi syndrome	●	●								●	●			●	Pearson-like syndrome	*LSMD*
12	M	1.5	8.1	Fanconi syndrome		●	●			●	●			●	●	●	●	●	KSS	*LSMD*
13	F	1	14.9	Fanconi syndrome		●	●				●		●		●	●	●	●	KSS	*LSMD*
14	F	0.1	1.5	Tubulopathy, renal diabetes insipidus		●	●				●		●	●	●		●	●	Pearson syndrome	*LSMD*
15	M	2	8.25	Increased amino acids in urine		●					●		●		●	●	●	●	KSS	*LSMD*
16	F	2	3.3	Hypokalemia, increased lactate and 3-MGA in urine		●	●				●		●	●	●	●	●	●	Complex I deficiency	*LSMD*
17	F	5.5	11.7	Increased albumin, glucose, and lactate in urine		●	●			●	●		●		●	●		●	KSS	*LSMD*
18	M	1.5	7	Hypochloremic metabolic alkalosis		●					●				●			●	KSS	*LSMD*
19	M	1	3.4	Fanconi syndrome		●	●				●		●					●	Complex III deficiency	*BCS1L*
20	F	0.25	1.4	Increased creatinine		●	●				●		●		●	●		●	COX 10	*COX10*
21	M	0	15	Increased protein in urine		●	●				●		●	●	●	●			Complex I and IV deficiency	*MTO1*
22	M	8	12.5	Hypertension, increased creatine and cystatin C			●			●	●	●						●	Nephronophthisis-like nephropathy	*XPNPEP3*

Table 1 (continued)

ID	Gender	Age at mito onset, years	Age at kidney onset, years	First renal manifestations	AKI	Tubulopathy	CKD stage 2 or greater	Nephrotic syndrome	Nephritic syndrome	Hypertension	CNS (encephalopathy, epilepsy)	PNS (peripheral neuropathy)	Muscle weakness or muscle hypotonia	Exercise intolerance	Ocular manifestations	Cardiac manifestations[§]	Diabetes mellitus	Abnormal growth	Mitochondrial phenotype	Genetics
23	F	12.3	14.5	Hypertension			●			●	●	●		●					Nephronophthisis-like nephropathy	*XPNPEP3*
24	F	0.7	7.8	na			●		●	●			●					●	RMND1	*RMND1*
25	F	0.5	5	Hypertension, increased protein in urine			●	●		●	●							●	RMND1	*RMND1*
26	M	20	65	Increased cystatin C			●			●	●		●		●			na	CPEO plus	*POLG1*
27	F	31.3	54	Increased creatinine			●				●	●	●		●				MELAS	*MTTL1*
28	M	46.4	53	Increased creatinine			●			●						●	●		MIDD	*MTTL1*
29	F	12	32.6	Fatigue			●		●	●		●			●				MELAS/LHON	*MTND5*
30	M	7	15.6	Increased creatinine and urea			●	●		●	●			●	●	●		●	Complex I + IV deficiency	*MTTR*
31	F	6	14.7	Increased urea, increased lactate and protein in urine			●				●	●	●	●	●	●			Complex I + IV deficiency	*MTTL1*
32	M	47	67	Increased cystatin C			●				●			●	●	●	●		MERRF	*MTTK*
33	F	0.3	12.3	Increased creatinine and urea; increased lactate 3-MGA and protein in urine			●				●		●		●	●	●	●	Pearson/KSS	*LSMD*
34	M	1	16	Renal parenchymal changes on ultrasound							●	●	●		●	●		●	Pearson/KSS	*LSMD*
35	F	2.25	5	Increased glycine in urine			●				●		●		●	●	●	●	KSS	*LSMD*
36	F	25.1	39.75	Increased creatinine			●				●				●	●	●		KSS	*LSMD*

Patients 1–7: renal manifestations as first manifestations of mitochondrial disease. Patients 5–11: patients with AKI. Patients 22–36: patients without AKI or tubulopathy. Patient 7 published in Freyer et al. [21]. Patient 8 published in Sofou et al. [22]. Patient 34 published in Komulainen et al. [23]. Patients 1, 22, and 23 published in O'Toole et al. [24]. M, male; F, female; 3-MGA, 3-methylglutaconic acid; na, not applicable; LSMD, large-scale mtDNA deletions. [§]Cardiac manifestations: 10 patients had arrhythmias (p2, 12, 13, 15, 17, and 32–36), 5 of them necessitating pacemaker. Seven patients had cardiomyopathy (p7, 16, 20, 21, 28, 30, and 31).

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Table 2. Findings from renal imaging, renal and muscle biopsy, and respiratory chain enzyme activity (n = 36)

ID	Imaging at renal onset (type of imaging)	Imaging later, if different (type of imaging)	Renal biopsy	Muscle biopsy	Respiratory chain enzyme activities	Mitochondrial phenotype	Genetics
1	Increased parenchymal echogenicity. Left cortical cyst (U)	NA	ND	ND	ND	Nephronophthisis-like nephropathy	*XPNPEP3*
2	Normal (urography)	Increased echogenicity, calcifications (X-ray)	Focal segmental glomerulosclerosis and general tubulopathy	RRF; COX deficient fibers 5–10%; lipid accumulation; enlarged mitochondria with abnormal cristae	Normal	KSS	*LSMD*
3	ND	ND	Focal segmental glomerulosclerosis	ND	ND	Nephronophthisis-like nephropathy	*MTTL1*
4	ND	Normal (U)	ND	RRFs	NA	MELAS	*MTTL1*
5	Extensive calcification/deposition in kidney pyramids (U)	NA	ND	ND	Complex III deficiency	Complex III deficiency	*BCS1L*
6	ND	ND	ND	ND	Complex III deficiency	Complex III deficiency	*BCS1L*
7	Increased echogenicity, bilateral renal dysplasia	Normal (U)	ND	Neurogenic abnormalities; groups of small fibers; EM normal	NA	CoQ10 deficiency	*CoQ7*
8	ND (normal at mito onset, but was performed before renal onset)	ND	Focal segmental glomerulosclerosis	Increased fiber caliber variation; structurally abnormal mitochondria on EM	Generalized deficiency	Alpers syndrome	*NARS2*
9	Left renal atrophy (U)	NA	ND	COX-neg fibers	ND	MELAS	*MTTL1*
10	Calcifications in parenchymal and sinus areas (U)	NA	ND	Nonspecific myopathy, type I fiber atrophy, EM normal	Complex I deficiency	Complex I deficiency	*MTND1*
11	ND (normal at mito onset, but was performed before renal onset)	ND	ND	ND	ND	Pearson-like syndrome	*LSMD*
12	Normal (urography)	Nephrocalcinosis (U)	ND	COX deficiency, mild steatosis	Complex IV deficiency	KSS	*LSMD*
13	Increased echogenicity (U)	NA	ND	RRF	Complex I deficiency	KSS	*LSMD*
14	Enlarged kidneys, increased echogenicity (U)	NA	NA	NA	Generalized deficiency	Pearson syndrome	*LSMD*
15	ND	ND	ND	Normal	Complex I deficiency	KSS	*LSMD*
16	Increased echogenicity	Small kidneys	ND	RRF; COX-neg fibers	Complex I deficiency	Complex I deficiency	*LSMD*
17	ND	Renal dysplasia	ND	RRF COX-neg fibers	Complex I deficiency	KSS	*LSMD*
18	Normal (U)	Left hydronephrosis	ND	RRF, COX-neg fibers, steatosis	Normal	KSS	*LSMD*
19	Normal (U)	Nephrocalcinosis	ND	Nonspecific type I fiber atrophy	Complex III deficiency	Complex III deficiency	*BCS1L*
20	Abnormal echogenicity (U)	NA	Nephrocalcinosis; periglomerular fibrosis: focal segmental glomerulosclerosis	COX-neg fibers, steatosis	Complex IV deficiency	COX 10	*COX10*
21	ND	ND	ND	Mitochondrial myopathy with accumulation of abnormal mitochondria (abnormal cristae, osmiofila inclusions)	Complex I and IV deficiency	Complex I and IV deficiency	*MTO1*

Table 2 (continued)

ID	Imaging at renal onset (type of imaging)	Imaging later, if different (type of imaging)	Renal biopsy	Muscle biopsy	Respiratory chain enzyme activities	Mitochondrial phenotype	Genetics
22	Increased parenchymal echogenicity. Bilateral cortical cysts (U)	NA	Nephronophthisis	ND	ND	Nephronophthisis-like nephropathy	*XPNPEP3*
23	ND	Small kidneys. Increased parenchymal echogenicity (U)	Nephronophthisis	Normal	Normal	Nephronophthisis-like nephropathy	*XPNPEP3*
24	ND	ND	ND	Congenital fiber type disproportion	Complex I, III and IV deficiency	RMND1	*RMND1*
25	Normal (U)	ND	ND	Predominance of type 1 fibers, slight lipid accumulation	Complex I, III and IV deficiency	RMND1	*RMND1*
26	Small kidneys (U)	NA	ND	NA	NA	CPEO plus	*POLG1*
27	Normal (U)	ND	ND	Abnormal	NA	MELAS	*MTTL1*
28	ND	Normal (U)	ND	NA	ND	MIDD	*MTTL1*
29	Normal (U, CT)	Small kidneys (U)	ND	RRF	Complex I deficiency	MELAS/LHON	*MTND5*
30	Small kidneys, increased echogenicity (U, xX-ray)	NA	ND	Mitochondrial proliferation, RRF, COX-neg fibers	Complex I + IV deficiency	Complex I + IV deficiency	*MTTR*
31	ND	Increased echogenicity (U); small kidneys, decreased tubular and parenchymal function (DMSA)	ND	Mitochondrial proliferation, RRF, some COX-neg fibers. On EM abnormal mitochondria with inclusions	Complex I + IV deficiency	Complex I + IV deficiency	*MTTL1*
32	ND	ND	ND	RRF, COXneg fibers 20%	NA	MERRF	*MTTK*
33	Normal (CT)	ND	ND	ND	ND	Pearson/KSS	*LSMD*
34	Increased echogenicity and parenchymal changes (U)	NA	ND	COX deficiency, RRFs, abnormal cristae of mitochondria	Complex I and III deficiency	Pearson/KSS	*LSMD*
35	ND	ND	ND	RRF; COX deficiency 20–30% of fibers at first-lipid accumulation and enlarged mitochondria with abnormal cristae	Generalized deficiency	KSS	*LSMD*
36	Normal (U)	ND	ND	NA	ND	KSS	*LSMD*

Patients 1–7: renal manifestations as first manifestations of mitochondrial disease. Patients 5–11: patients with AKI. Patients 22–36: patients without AKI or tubulopathy. Patient 7 published in Freyer et al. [21]. Patient 8 published in Sofou et al. [22]. Patient 34 published in Komulainen et al. [23]. Patients 1, 22, and 23 published in O'Toole et al. [24]. U, ultrasound; CT, computed tomography; ND, not done; NA, not available; RRF, ragged red fibers; LSMD, large-scale mtDNA deletions.

ed GFR (eGFR) using the creatinine-based bedside Schwartz equation [11]. For defining and staging chronic kidney disease (CKD), we used the Kidney Disease Improving Global Outcomes (KDIGO) guidelines [12]. CKD stage 2 or greater was defined as a GFR <90 mL/min/1.73 m^2. Acute kidney injury (AKI) was diagnosed clinically, relying on changes in serum creatinine and/or urine output, as proposed by KDIGO [13]. Increased excretion of albumin and total protein in urine was evaluated based on the KDIGO guidelines [13].

Nephrotic syndrome was defined as severe albuminuria (urine albumin/creatinine ratio >400 mg/mmol [or >3500 mg/g] or urine albumin >50 mg/kg/24 h), hypoalbuminemia (serum albumin <25 g/L), and edema. For the diagnosis of nephritic syndrome, the patient had to fulfill all of the following: (a) hematuria (≥2+ on urine dipstick analysis) with or without proteinuria, (b) increased serum creatinine adjusted for age, and (c) hypertension (systolic or diastolic arterial pressure ≥95th percentile adjusted for age and height).

Proximal tubulopathy, i.e., dysfunction of the renal proximal tubules, was defined as having one or more of the following: low-molecular-weight proteinuria, general aminoaciduria, glucosuria (with normal plasma glucose levels), bicarbonate loss resulting in renal tubular acidosis, and electrolyte wasting. The combination of bicarbonate loss with variable renal wasting of amino acids, glucose, phosphate, uric acid, and other solutes was defined as renal Fanconi syndrome. Renal Fanconi syndrome with hypophosphatemic rickets and glycosuria was defined as de Toni-Debré-Fanconi syndrome [14]. Isolated distal tubulopathy was defined as urinary wasting of electrolytes (Na^+, K^+, and Cl^-) in the absence of signs of proximal tubular dysfunction, while coexistence of proximal and distal tubulopathy was characterized as generalized tubulopathy.

Protocol Development

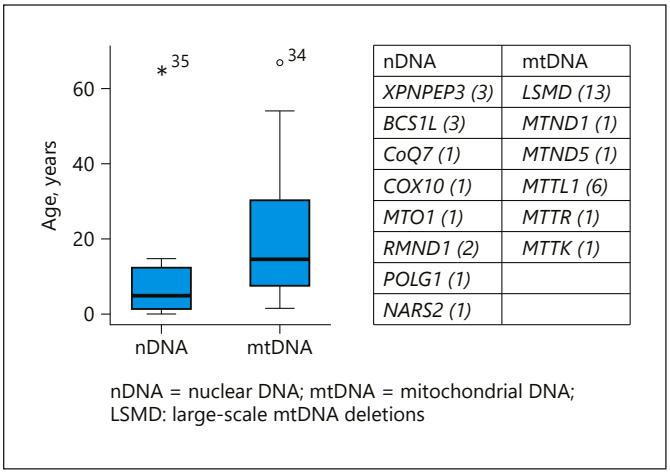
We developed a protocol for the assessment and monitoring of renal function in mitochondrial disease patients prior to established kidney disease. Development of this protocol relied on results from this study and the consensus for patient care standards [15] and was critically reviewed by all MCRN investigators who participated in this study.

Statistical Considerations

The statistical evaluations performed were mainly exploratory. Survival outcomes were estimated with the help of Kaplan-Meier analysis. The χ^2 test was used to test the association between categorical variables. The independent *t* test was used to test differences between categorical variables, such as gender, and continuous variables, such as age at onset. All statistical tests were 2-sided and performed at a 0.05 significance level.

Results

A total of 36 patients were included (19 males and 17 females). The majority had mtDNA-associated disease (23/36). The median age at onset of mitochondrial disease was 2 years (prenatal–47 years of age). Patients were followed for a median period of 15 years (0.1–49.5 years).



nDNA	mtDNA
XPNPEP3 (3)	*LSMD (13)*
BCS1L (3)	*MTND1 (1)*
CoQ7 (1)	*MTND5 (1)*
COX10 (1)	*MTTL1 (6)*
MTO1 (1)	*MTTR (1)*
RMND1 (2)	*MTTK (1)*
POLG1 (1)	
NARS2 (1)	

Fig. 1. Age at renal disease onset in patients with mtDNA versus nDNA-associated disease ($p = 0.062$ for the entire group; $p = 0.011$ when excluding the 2 extreme values of ID 34 [=67yo] and ID 35 [=65yo]). A list of underlying genetics is presented with the corresponding number of patients in parenthesis. LSMD, large-scale mtDNA deletions.

Onset of Renal Manifestations

The median age at onset of renal manifestations was 12 years (birth–67 years). Renal manifestations occurred earlier in patients with nDNA-associated mitochondrial disease (nDNA 5 y; mtDNA 15 y; $p = 0.011$; Fig. 1). A summary of patients' age at disease onset, renal phenotype, mitochondrial extrarenal manifestations, and genotype is shown in Table 1.

Renal manifestations were the first sign of mitochondrial disease (ID1-7 in Table 1) in 7 patients, either alone (ID1, 4, and 5) or in combination with anemia (ID2), hearing impairment (ID3), or epilepsy (ID6). One patient had multiorgan disease at birth, which, apart from renal manifestations, included lung hypoplasia, persistent pulmonary hypertension, systemic arterial hypertension, and secondary left ventricle hypertrophy (ID7). Three of these 7 patients presented with AKI in the first month of life. In the patient with multiorgan involvement (ID7), renal manifestations resolved spontaneously by the first year of life and had not reappeared by the last follow-up visit at 11 years of age.

Renal involvement was first captured by laboratory tests, either as part of routine workup or fortuitously, in 20 patients (20/35; 1 unknown) (Table 1). Isolated increased levels of serum creatinine or cystatin C were the presenting feature in 9 patients (9/35; 1 unknown).

Fifteen patients presented with symptoms such as fatigue, polydipsia, polyuria, and hypertension, either alone

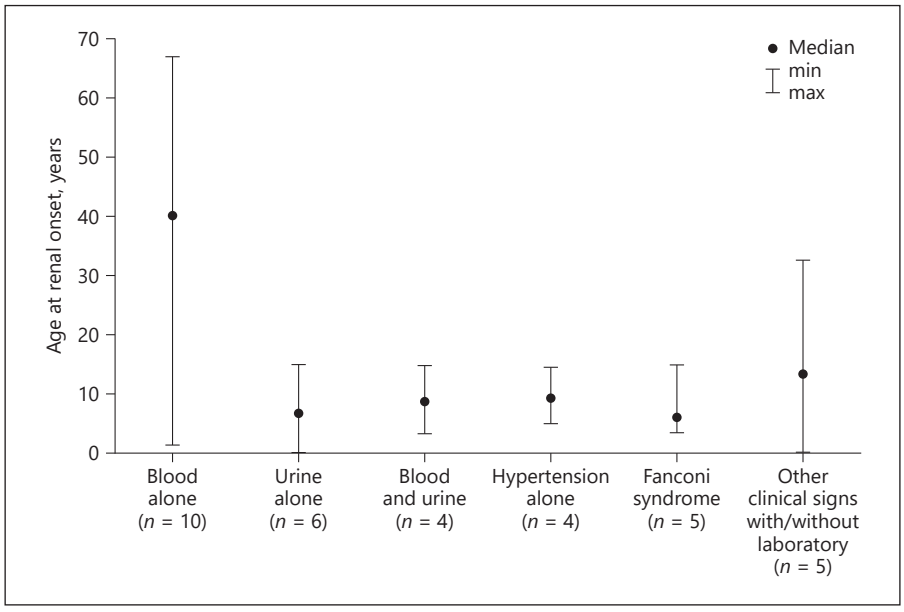


Fig. 2. Age when the first renal manifestations occurred, grouped by type of renal manifestations ($n = 34/36$; 1 with abnormal renal imaging and 1 unknown), i.e., abnormal renal function tests in blood alone ($n = 10$), abnormal renal function tests in urine alone ($n = 6$), abnormal renal function tests in blood and urine without clinical signs ($n = 4$), arterial hypertension alone ($n = 4$), Fanconi syndrome ($n = 5$), and clinical signs or symptoms of renal disease with or without arterial hypertension ($n = 5$).

or in combination with abnormal laboratory tests, and/or abnormal renal imaging. Fanconi syndrome was the presenting feature in 5 patients (5/35; 1 unknown).

The identification of renal disease on routine laboratory testing or fortuitously occurred in all age groups and, as expected, tended to occur later than in those with clinical signs of renal disease ($p = 0.108$) (Fig. 2). No correlation was found between the type of presenting renal manifestations and gender, genetic background, or renal disease outcome.

Acute Kidney Injury

Seven of 36 patients developed AKI (ID5-11, Table 1) at a median age of 4 years (birth–46 years), and in 5, AKI was the first manifestation of renal disease. CKD preceded AKI in 1 patient (ID9). No repeated episodes of AKI were seen in any of our patients. The occurrence of tubulopathy was common among patients with a history of AKI (5/7). Four of 7 patients developed Fanconi syndrome, of whom one fulfilled the criteria for de Toni-Debré-Fanconi syndrome (ID11). No correlation was found between the history of AKI and the gender, age at onset of renal disease, or mitochondrial genetics. Rhabdomyolysis was not seen in any of the patients.

Other Renal Manifestations, Laboratory Findings, and Management

CKD with decreased GFR (stage 2 or greater) was found in 27 of 36 patients, as shown in Table 1. Twenty-two patients had a CKD stage 3 to 5 by the time of last follow-up (median age 26.35 years old, min–max: 5.75–69.5). The incidence of CKD was equally distributed across gender, age at renal onset, and genetic background. Seven patients underwent renal replacement therapy, i.e., dialysis (4) and/or renal transplantation (3). The age at start of dialysis was 19 years (min–max: 18–20 years; 1 unknown). Dialysis was initiated at a median of 13 years after onset of renal manifestations (min–max: 5–16 years; 1 unknown). One of the 4 patients with dialysis received a renal transplant shortly afterward, while another patient died 2 months after initiation of dialysis. One patient was on dialysis for 3 years, until death due to cardiac complications.

Tubulopathy was found in 16 patients (16/36): Fanconi syndrome (7/16), proximal tubulopathy (4/16), generalized tubulopathy (1/16), and unspecified (4/16). Tubulopathy was significantly more common among patients with large-scale mtDNA deletions, compared to patients with other genetic etiologies ($p = 0.025$).

Hyperlactatemia was found at least once in 27 patients (27/32; unknown in 4 patients). An increased excretion of lactate and/or 3-methylglutaconic acid in urine was common (13/18), while multiple organic acidurias were seen in 3 patients. General aminoaciduria was seen in 10 of 24 patients, while 2 patients, both with KSS due to large-scale mtDNA deletions, had repeatedly increased excretion of glycine in urine (ID18 and 35). Increased excretion of electrolytes in urine and/or low plasma levels necessitating electrolyte supplementation was found in 17 patients (17/36).

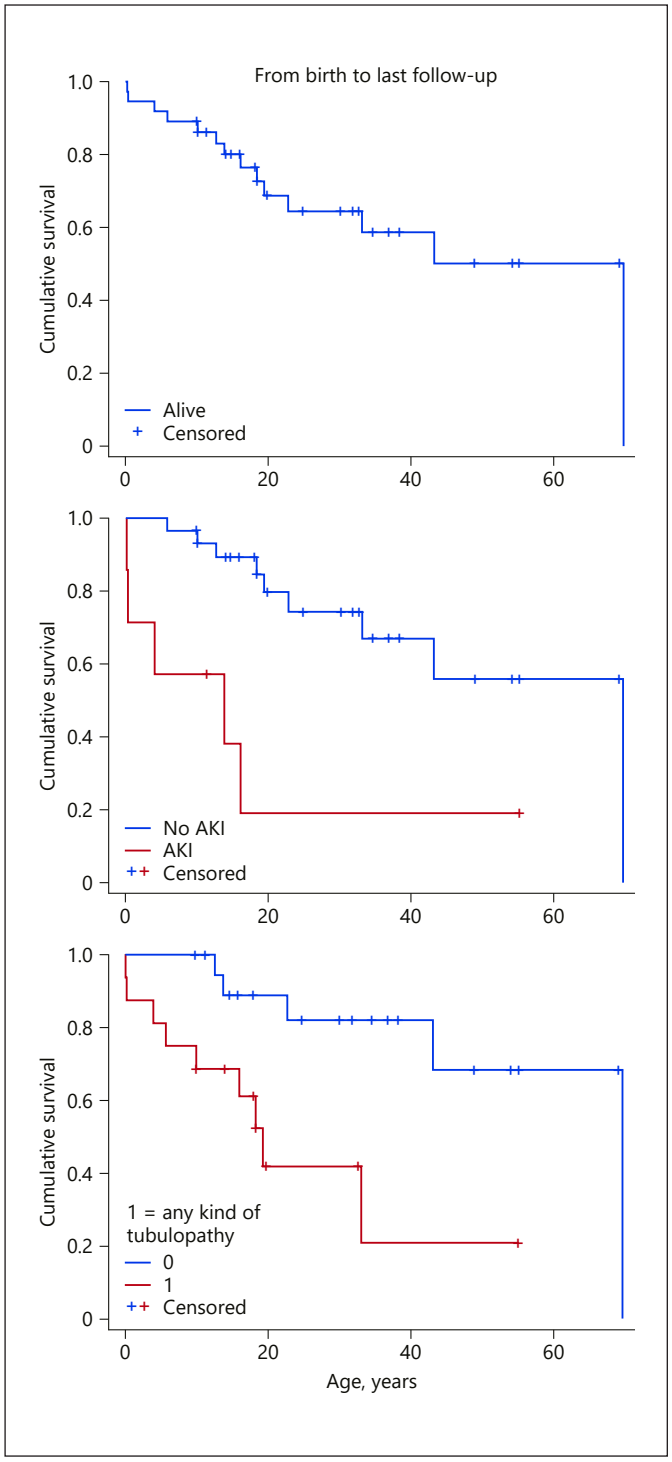


Fig. 3. Survival curves for the entire cohort ($n = 36$; **a**), acute kidney injury ($n = 7/36$; **b**) versus the rest of the cohort, and any kind of tubulopathy ($n = 16/36$; **c**) versus the rest of the cohort.

Hypertension was found in 12 patients (12/36) and was the presenting feature in 4 of them; i.e., in 3 XPNPEP-patients (ID1, 22, and 23) and in 1 RMND1-patient (ID25). Nephrotic syndrome was found in 4 patients and nephritic syndrome in 2 patients (Table 1).

Renal Imaging and Biopsy Findings

Renal imaging was performed in 29 patients (29/36) and was abnormal in 21. In 5 of 21, renal imaging was initially normal. Table 2 summarizes the renal imaging and biopsy findings, along with the results from the respiratory chain enzyme analyses. The most common findings on renal imaging were increased echogenicity (12/21), renal dysplasia/hypoplasia (9/21), calcifications/nephrocalcinosis (5/21), and renal cysts (2/21). Renal biopsy was performed in 6 patients and was abnormal in all. Main renal biopsy findings were focal segmental glomerulosclerosis, nephrocalcinosis, and nephronophthisis (Table 2).

Survival Outcome

Fourteen of 36 patients died at a median age of 15 years (0.1–69.5 years) (Fig. 3). A history of AKI or tubulopathy correlated with reduced survival ($p = 0.002$ and $p = 0.005$, respectively) (Fig. 3). No other specific renal phenotype correlated with patients' survival outcomes, nor was there any correlation to the underlying type of genetic defect (mtDNA vs. nDNA-associated disease, $p = 0.568$).

In our study, 5 of 7 patients with AKI died at a median age of 4 years (0.1–13.75 years), 4 of them within 6 months after the onset of AKI. None of these patients suffered from cardiovascular, pulmonary, or endocrinological manifestations other than abnormal growth.

Discussion

Renal function is highly dependent on aerobic respiration, making it vulnerable to energy deprivation. Defects in mitochondrial energy metabolism have been associated with various renal manifestations, including proximal tubulopathy and CKD. Our results emphasize the occurrence of AKI as a distinct renal phenotype in patients with mitochondrial disease. We further elaborate on clinical indicators of isolated renal disease suggestive of underlying mitochondrial disease.

AKI is characterized by an abrupt decrease in the GFR. Mitochondrial damage including mitochondrial fragmentation, swelling, and loss of inner structure contribute critically to AKI development [16]. Mitochondrial

Table 3. Protocol for the assessment and monitoring of renal function in mitochondrial disease patients without known renal involvement (presymptomatic monitoring)

Blood pressure at least yearly
Blood and urine assessment yearly and at episodes of acute decompensation, as follows
Blood: creatinine, cystatin C, urea, sodium, potassium, calcium, magnesium, phosphate, lactate, albumin, uric acid
Urine: U-dipstick, pH, uric acid, amino acids, organic acids, u-albumin/creatine ratio or u-protein/creatinine ratio, electrolytes, U-low-molecular-weight proteins (U-α1-MG, U-β2-MG)
Renal imaging (preferably ultrasound) at diagnosis of mitochondrial disease and repeat upon indication (e.g., decreased GFR and hypercalciuria)
Upon clinical, laboratory, or radiological signs of kidney involvement, contact with nephrologist, decision on complementary imaging and/or renal biopsy
In mitochondrial patients with high risk of developing kidney disease (e.g., patients with large-scale mtDNA deletions), consider contact with nephrologist early in the disease course

U-α1-MG, urine alpha-1-microglobulin; U-β2-MG, urine beta-2-microglobulin.

oxidative stress and decreased adenosine triphosphate production primarily affect the renal tubular cells in AKI [16]. Mitophagy and mitochondrial biogenesis are 2 renoprotective mechanisms with a substantial role in the recovery phase of AKI [16, 17]. If these mechanisms are compromised, as in patients with genetic mitochondrial disease, the kidneys have limited recovery capacity after AKI, which can explain why most of our patients with AKI developed renal Fanconi syndrome.

More than half of our AKI patients died shortly after AKI, with no preceding signs of multiple organ involvement. AKI is known as an important precipitant of end-stage renal failure. The 5-year mortality after AKI is high even among patients treated with renal replacement therapies [18]. Our results suggest that AKI is important to recognize as it suggests decompensating mitochondrial disease and poor short-term survival outcome. Increased awareness among mitochondrial disease physicians is therefore vital, as early detection and initiation of prevention, including hydration and removing nephrotoxic drugs, are the mainstays of treatment.

AKI was the first manifestation of mitochondrial disease in 3 of our patients, either alone or in combination with other clinical signs or symptoms. Other renal manifestations in previously undiagnosed patients were proximal tubulopathy, with lactic aciduria and/or generalized aminoaciduria, renal Fanconi syndrome, arterial hypertension, and kidney dysplasia. Whilst we recognize that there are more common causes, our findings suggest that an underlying mitochondrial disease should be considered in patients with (i) proximal or generalized tubulopathy especially in the presence of lactic aciduria or generalized aminoaciduria, (ii) unexpected AKI in whom common causes are absent, or (iii) arterial hypertension in children, adolescents, or young adults in the absence of risk factors for primary hypertension. When these renal manifestations are preceded by systemic infection or other metabolic stress, occurring as part of a multiorgan involvement, or when at least 2 of the abovementioned manifestations concur, the suspicion of underlying mitochondrial dysfunction is warranted.

Renal involvement was first captured on laboratory tests, either as part of routine workup or incidentally in 57% of our patients. In half of these patients, increased creatinine and/or cystatin C levels in serum were the only sign. It is, therefore, likely that patients with mitochondrial disease, and especially those with multisystemic involvement, have renal involvement that will remain undetected unless actively sought after. Hence, prudent and preplanned monitoring of renal function should be part of routine follow-up of patients with mitochondrial disease. In our protocol for the assessment and monitoring of renal function in mitochondrial disease patients without known renal involvement, we propose a laboratory assessment of renal function yearly and upon episodes of acute metabolic decompensation, shown in Table 3. As cystatin C appears to have a higher diagnostic accuracy than creatinine in assessing GFR among patients with mitochondrial defects [19], it should be included in the routine follow-up. Since we show that normal renal imaging does not exclude renal disease, we propose that renal imaging is performed at diagnosis of mitochondrial disease and repeated when renal manifestations occur. Renal biopsy is an invasive procedure and should be reserved when diagnostic difficulties occur or for monitoring of specific treatments.

One limitation of this study was that, based on the definition criteria used for nephritic syndrome, mild forms of nephritic syndrome may have been missed. Another limitation was the use of different biomarkers, such as se-

rum creatinine, serum cystatin C, or both, to evaluate renal function. As direct measurement of GFR either by 99mTc-DTPA (diethylenetriaminopenta-acetic acid), 51Cr-EDTA (ethylenediaminetetra acetic acid), or iohexol is complicated and expensive, GFR is usually estimated using an endogenous biomarker, most commonly serum creatinine or cystatin C, in order to calculate an eGFR using the Schwartz or other equations [20]. However, all these indirect methods to estimate GFR are vulnerable to several types of bias arising, e.g., from hyperfiltration states, patient's sex, race, diet, age (for creatinine-based estimations), various diseases such as thyroid disease, low muscle mass (for creatinine), and high-dose corticosteroid treatment (for cystatin C). It is also worth mentioning that due to lack of standardization of the 2 most common assays to measure cystatin C, in each GFR estimation equation, the same method that was used for cystatin C measurement in the equation should be applied [20].

Our study shows that AKI is a distinct renal phenotype in mitochondrial diseases, and that it is associated with poor disease outcome. Early and prompt recognition of AKI and of renal disease in general is crucial for optimal patient management. A protocol for the assessment of renal function is proposed to help physicians to systematically monitor their patients. Early detection of renal symptoms and signs in a mitochondrial disease patient will facilitate a better usage of the available therapeutic arsenal and improve quality of life of the patient and reduce long-term complications and treatment costs.

Statement of Ethics

Our study complied with the ethical guidelines and was conducted in accordance with the World Medical Association Declaration of Helsinki. The study protocol was approved at each participating center. As this was a retrospective study of de-identified patient data, the need for informed consent was waived by the regulatory authorities (Ethics Committee Etikprövningsmyndigheten, Sweden [previously Regionala Etikprövningsnämnden Göteborg]; METC, Rotterdam; The Ethics Committee of Northern Ostrobothnia Hospital District, Oulu; The Ethics Committee of Helsinki University Hospital, Helsinki; Regional Ethics Committee, Bergen; Ethics and Research Committee of the Fundación Sant Joan de Déu, Institut de Recerca Hospital Sant Joan de Déu, Barcelona; Danish Patients Safety Authority and the Capital Region of Denmark).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

All authors equally contributed to study conception and design, data acquisition, and critical review of the results and of the manuscript. Maria Parasyri and Kalliopi Sofou also performed data management and statistical analysis as well as drafting of the manuscript. Kalliopi Sofou and Niklas Darin share co-last authorship.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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