Pediatr Nephrol (2000) 14:495–498

BRIEF REPORT

Eberhard Kuwertz-Bröking · Hans Georg Koch
Thorsten Marquardt · Rainer Rossi · Udo Helmchen
Josef Müller-Höcker · Erik Harms · Monika Bulla

Renal Fanconi syndrome: first sign of partial respiratory chain complex IV deficiency

Received: 6 October 1998 / Revised: 17 August 1999 / Accepted: 18 August 1999

Abstract A 2-year-old boy who developed hypophosphatemic rickets without signs of muscular weakness or neurological disturbances is presented. Biochemical findings included hypophosphatemia, metabolic acidosis, hypouricemia, hyperphosphaturia, severe glucosuria, generalized hyperaminoaciduria, hypercalciuria, proteinuria with elevated excretion of IgG, transferrin, albumin and high levels of α-1-microglobulin. Urine concentration capacity and creatinine clearance were normal. Lactaturia without elevated levels of plasma lactate and a high urinary excretion of β-hydroxybutyrate were suggestive for mitochondriopathy. Partial deficiency of cytochrome *c* oxidase (complex IV of the respiratory chain) was found in skeletal muscle. A renal biopsy specimen demonstrated enlarged mitochondria with abnormal arborization and disorientation of the cristae in the proximal tubular cells. Reduced activity of mitochondrial cytochrome *c* oxidase in tubular cells could be demonstrated by ultracytochemistry. In conclusion, rickets due to the renal Fanconi syndrome can be the first clinical sign of mitochondrial cytopathies without extrarenal symptoms. Elevated excretion of lactate and ketone bodies in urine may serve as a diagnostic marker.

Key words Rickets · De Toni-Debré-Fanconi syndrome · Lactaturia · Mitochondriopathy · Cytochrome *c* oxidase deficiency

E. Kuwertz-Bröking (✉) · H.G. Koch · T. Marquardt · E. Harms
M. Bulla
Department of Pediatrics, Pediatric Nephrology,
University Children's Hospital of Münster, Waldeyerstrasse 22,
48149 Münster, Germany
Tel.: +49-251-9813311, Fax: +49-251-9813336

R. Rossi
Children's Hospital Neukölln, Berlin, Germany

U. Helmchen
Department of Pathology, University of Hamburg, Hamburg, Germany

J. Müller-Höcker
Department of Pathology,
Ludwig Maximilians University München, München, Germany

Introduction

Hypophosphatemic rickets can be caused by the de Toni-Debré-Fanconi syndrome, characterized by generalized dysfunction of the proximal tubule. Different types of Fanconi syndrome can be classified according to their etiology and pathophysiology [1]. An increasing number of children with a more or less complete renal Fanconi syndrome due to mitochondrial cytopathies have been reported recently [2–7]. Many organs may be affected by defects in the mitochondrial respiratory chain. Usually, children with these disorders present with mitochondrial encephalomyopathies, whereas renal involvement is not a predominant symptom [8, 9]. We report a patient with hypophosphatemic rickets as the first clinical sign of a renal Fanconi syndrome caused by a partial deficiency of cytochrome *c* oxidase (complex IV) of the mitochondrial respiratory chain.

Case report

The patient was admitted to a hospital at the age of 2 years because of inguinal hernia. Routine blood chemistry before surgery demonstrated hypophosphatemia (0.71 mmol/l) and elevated alkaline phosphatase activity (5269 U/l). The boy's length was between 3rd and 10th percentiles. Clinical examination demonstrated typical signs of rickets with genu varum and expansion of metaphyses in wrists and knee joints. Neurological examination was normal and there were no signs of muscular hypotonia. The parents reported the child's complaints of pain in the legs especially after walking longer distances.

Laboratory investigations (Table 1) revealed a generalized dysfunction of the proximal tubule: decreased fractional phosphate reabsorption (phosphate reabsorption related to creatinine clearance, Tp/C_{Crea}), low values of percent glucose reabsorption (% T_{Gluc}), urinary losses of bicarbonate, uric acid, and generalized hyperaminoaciduria. Hyperaminoaciduria was seen in 15 of 16 amino acids (%TAA: 64.3–99.1). Hypercalciuria accompanied by medullary hyperechogenicity was seen upon ultrasonic kidney evaluation (Fig. 1). Unselective glomerular proteinuria (albumin, IgG, transferrin) and tubular proteinuria (α-1-microglobulin) and an increased excretion of lactate and β-hydroxybutyric acid in urine were also demonstrated. Urine concentration ability was normal. Blood chemistry showed severe metabolic acidosis, hypouricemia,

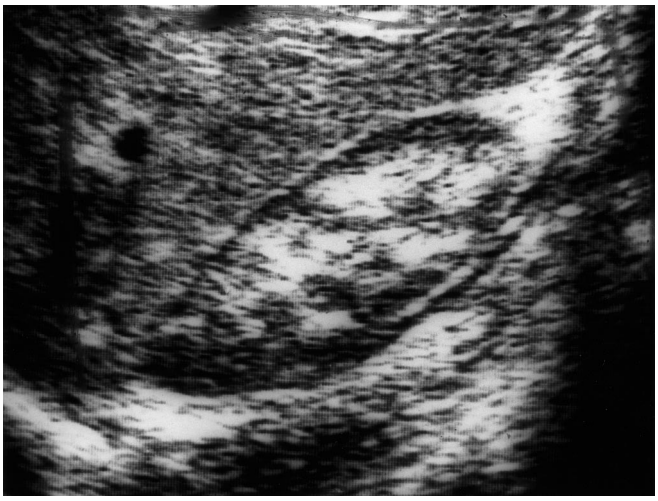

Fig. 1 Ultrasound of the right kidney

Table 1 Blood and urine investigations (*% T_{Gluc}* percent glucose reabsorption, *Tp/C_{Crea}* phosphate reabsorption related to creatinine clearance)

		Patient	Controls
Blood			
Calcium	(mmol/l)	2.5	2.1–2.7
Inorganic Phosphorus	(mmol/l)	0.71	1.1–2.0
Alkaline Phosphatase	(U/l)	5269	128–593
Creatinine	(µmol/l)	53.0	27–62
Creatinine clearance	(ml/min/ 1.73 m^2)	101	89–165
Urea nitrogen	(mmol/l)	4.3	2–7
Bicarbonate	(mmol/l)	15.6	22–26
Base excess		–8	±2
Uric acid	(µmol/l)	47	120–420
Parathormone (intact)	(pg/ml)	165	9–62
25-OH-Vit.-D3	(mmol/l)	13	50–300
1,25-$(OH)_2$-Vit.-D3	(ng/l)	52	35–90
Lactate	(mmol/l)	0.52	0.5–2.0
β-Hydroxybutyrate	(µmol/l)	25	<100
Urine			
% T_{Gluc}	(%)	46	>99.7
Tp/C_{Crea}	(µmol/ml)	1.04	1.5 (±0.22)
Calcium/creatinine	(mol/mol)	2.6	<1.5
Total protein	(mg/l)	690	15–130
Protein/creatinine	(mg/mg)	2.4	<0.1
Albumin	(mg/l)	343	8–20
IgG	(mg/l)	134	1–3
Transferrin	(mg/l)	62	0.5–1.3
α-1-microglobulin	(mg/l)	610	3–8
Lactate/creatinine	(molar ratio)	1.09	<0.017 (*)
β-Hydroxybutyrate	(mmol/l)	8.98	<1.0 (*)

(*) Reference values from our laboratory

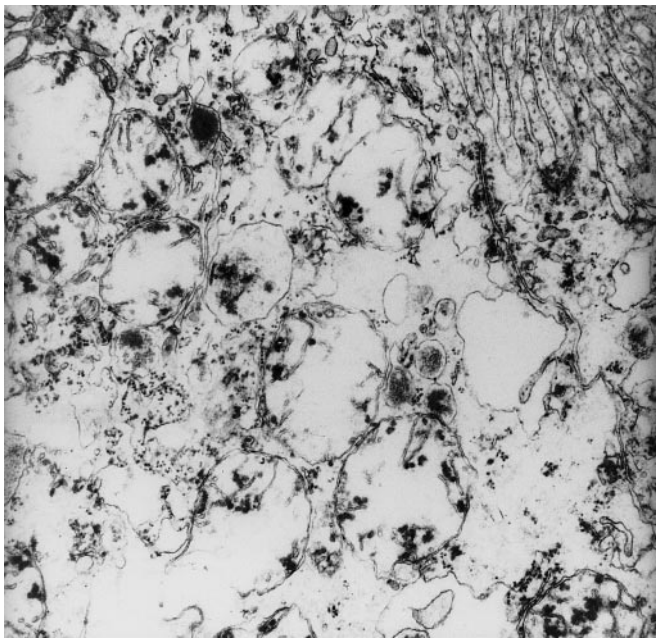

Fig. 2 Electron microscopy of proximal tubular cells. Proximal tubular cells with normal brush border, enlarged mitochondria and disorientation of the cristae (×4700)

mild hyperparathyroidism, and mildly decreased levels of 25-OH-vitamin D3. Magnesium levels, liver function parameters, lactate, and pyruvate levels and lactate/pyruvate molar ratios in plasma as well as amino acid values were normal. Radiography of the wrists and the knee joints proved rickets.

Treatment consisted of oral administration of sodium bicarbonate (3 mmol/kg per day), phosphorus (1000 mg/day), cholecalciferol (3000 U/day), hydrochlorothiazide (1.5 mg/kg per day), and citric acid (0.2 g/kg per day). With this regimen, alkaline phosphatase values dropped only to 2000 U/l, parathyroid hormone (PTH) levels to 102 pg/ml. Radiographic controls of the wrist 6 months after introduction of therapy showed only mild improvement of rickets. Elevated levels of lactate and β-hydroxybutyrate persisted in urine.

For further diagnostic clarification both renal and skeletal muscle biopsy were performed. Histopathological examination of the muscle specimen could not demonstrate pathological fibers or structural mitochondrial abnormalities. Low normal values of cytochrome *c* oxidase (complex IV of the respiratory chain) were found in muscle specimen. However, cytochrome *c* oxidase activity in relation to the activity of citrate synthase as a marker enzyme for the content of mitochondria demonstrated a partial complex IV deficiency (Table 2).

Microscopic examination of the kidney biopsy specimen revealed normal glomeruli and tubulo-interstitium, but ultrastructurally, enlarged mitochondria with abnormal arborization and disorientation of the cristae were found in the proximal tubular cells (Fig. 2). In these cells reduced activity of mitochondrial cytochrome *c* oxidase could be demonstrated by ultracytochemistry (Fig. 3).

Table 2 Activity of respiratory chain enzymes in skeletal muscle mitochondrial fractions. Measurements were done according to Paetzke et al. [10] and Trijbels et al. [11] (*NCP* non-collagen protein)

	Patient (U/g NCP)	Controls (U/g NCP)
NADH:CoQ oxidoreductase (complex I)	53.4	12.0–26.4
Succinate:cytochrome *c* oxidoreductase (complex II+III)	23.5	6.0–25.0
Cytochrome *c* oxidase (complex IV)	93.0	90.0–281.0
Citrate synthase	159.0	45.0–100.0
NADH:CoQ oxidoreductase/citrate synthase	0.34	0.17–0.56
Succinate:cytochrome *c* oxidoreductase/citrate synthase	0.15	0.08–0.45
Cytochrome *c* oxidase/citrate synthase	0.58	0.90–4.70

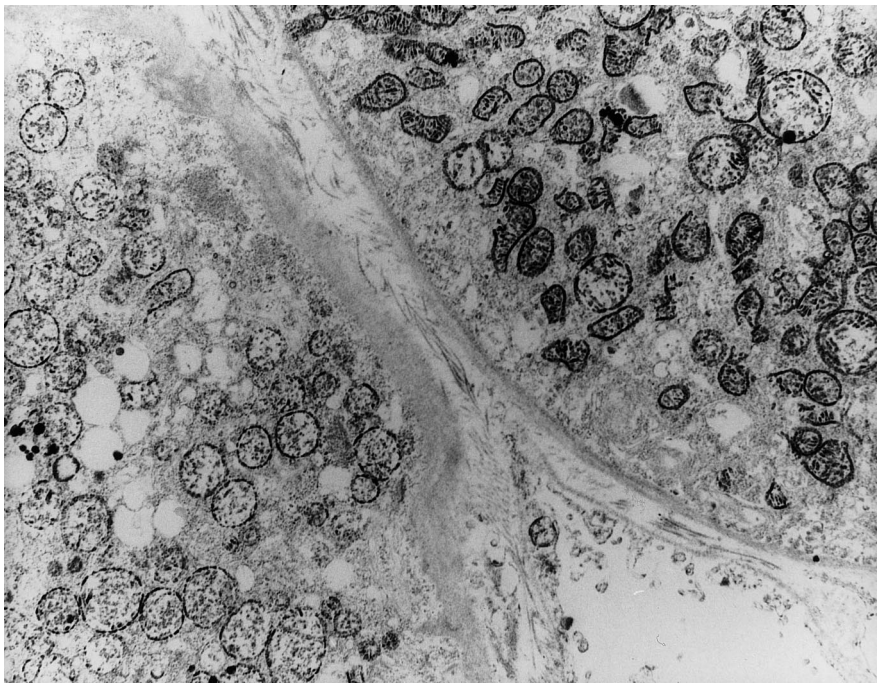

Fig. 3 Ultracytochemistry of cytochrome *c* oxidase in proximal tubular cells. There is a reduced decoration of the mitochondria in one tubule, whereas the adjacent tubule shows an intensive staining of the mitochondria by the reaction product (Diaminobenzidine ×5800)

Four years after diagnosis of mitochondrial dysfunction, no signs of involvement of other organ systems were observed. Proximal tubular dysfunction did not progress, serum creatinine values, GFR, and growth velocity remain within the normal range.

Methods

Mitochondrial enzyme activities were measured in fresh frozen muscle tissue taken from the four-head muscle. In a homogenate prepared from 40 mg muscle tissue, measurements of the enzyme activities were performed as described by Paetzke et al. [10] and Trijbels et al. [11].

For electron microscopical determination of the complex IV activity in the frozen kidney biopsy specimen the method as described by Müller-Höcker et al. [12] was applied. The tissue slices are incubated with a 3,3'-diaminobenzidine tetrahydrochloride (DAB) solution. DAB is oxidized by the enzyme and undergoes oxidative polymerization to produce water-insoluble material that is strongly osmiophilic. On postfixation with aqueous osmium tetroxide, a characteristic contrast on ultrastructural examination can be achieved. Defective cells lacking cytochrome *c* oxidase reaction product can be easily localized. Intracellular heterogeneity of the enzyme activity can also be detected.

Discussion

The clinical spectrum of defects of the respiratory chain is expanding. Usually, failure of organs that have a high oxidative metabolic demand is seen. Consequently brain and muscle dysfunction are particularly common [2, 5, 6, 9]. An increasing number of patients with renal involvement in mitochondrial cytopathies has been described in the literature in the last few years, but the de Toni-Debré-Fanconi syndrome as the first predominant symptom of a cytochrome *c* oxidase disorder several years before manifestation of mitochondrial encephalomyopathy has only been observed in a single case [3]. Mochizuki et al. in 1996 [3] reported one case of Kearns-Sayre syndrome diagnosed in an 11-year-old girl, which was preceded by a Fanconi syndrome 8 years before. At the age of 3 years, this girl was diagnosed as having a renal Fanconi syndrome. Three years later, diabetes and mild renal insufficiency was observed and kidney biopsy demonstrated glomerular sclerosis and severe tubular atrophy. Electron micrographs of distal tubular cells demonstrated enlarged mitochondria with circumferentially orientated cristae and an abundant, granular matrix. Mitochondria of the proximal tubules were normal.

In our patient the cause of renal Fanconi syndrome was unknown after our first examinations. Urinary levels of lactate and β-hydroxybutyrate were elevated constantly, while plasma lactate levels and lactate/pyruvate ratios were in the normal range. These findings were suggestive for a defect of the mitochondrial respiratory chain affecting the kidneys. A standardized procedure for the diagnosis of mitochondriopathy is the enzymatic analysis of respiratory chain complexes in muscle tissue. A disturbed cytochrome *c* oxidase activity (complex IV) in muscle specimen was shown in our patient. The ultrastructural examination of proximal tubular cells in the renal biopsy specimen, taken at the same time, demonstrated enlarged mitochondria with rarefaction and aberrant arborization of the cristae (Fig. 2) and decreased activity of cytochrome *c* oxidase using ultracytochemical staining reactions (Fig. 3). In contrast to the patient mentioned above [3], the mitochondria of distal tubular cells were normal.

Renal Fanconi syndrome is observed in patients with complex III and complex IV deficiency [2, 4–7]. Usually, lactic acidosis and pathological lactate/pyruvate mo-

lar ratios in plasma are demonstrated in these patients and renal Fanconi syndrome is an associated renal problem, while symptoms of encephalomyopathy are dominant. Our patient has been followed up for 4 years and the renal Fanconi syndrome is still the only clinical symptom of the mitochondrial respiratory chain defect. It is, however, still possible that dysfunction of muscle or brain will develop in the future.

In conclusion, the laboratory results of the plasma and urine tests, increased urinary excretion of lactate and ß-hydroxybutyrate despite normal blood values, morphological abnormalities of mitochondria of proximal tubular cells, focal cytochrome *c* oxidase deficiency in tubular ultracytochemical examination and partial deficiency of complex IV in muscle tissue in this patient point to a predominant renal manifestation of a mitochondrial cytopathy. Investigations of patients with renal Fanconi syndrome of unknown origin should include the determination of lactate and ketone bodies in urine. Elevated concentrations are suggestive for renal mitochondrial respiratory chain disorders.

Acknowledgements We would like to thank Pr. K. Gerbitz and Dr. I. Paetzke at the Department of Clinical Chemistry, Hospital München-Schwabing, for measurement of mitochondrial enzyme acitivities in four-head muscle. This paper was presented as a poster at the eleventh congress of the International Pediatric Nephrology Association in London (12–16 September 1998).

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LITERATURE ABSTRACT

B.M. Sibai · M. Lindheimer · J.Hauth · S. Caritis
P. VanDorsten · M. Klebanoff · C. MacPherson · M. Landon
M. Miodovnik · R. Paul · P. Meis · M. Dombrowski

Risk factors for preeclampsia, abruptio placentae, and adverse neonatal outcomes among women with chronic hypertension

N Engl J Med (1998) 339:667-671

Objective Risk factors for preeclampsia and adverse neonatal outcomes in pregnant women with chronic hypertension were identified.

Methods A group of 763 pregnant women with chronic hypertension (age distribution: 27% < 25 years, 28% 26-30 years, 45% >30 years) was prospectively followed from 13-26 weeks of gestation (mean: 20 weeks) to the end of pregnancy.

Results One hundred ninety-three (25%) had preeclampsia at baseline, a frequency not affected by the presence of baseline proteinuria (27% vs. 25%). Preeclampsia was significantly greater in women who had had hypertension for at least four years (31% vs. 22%) and in those with preeclampsia during a previous pregnancy (32% vs. 23%). Women with proteinuria at baseline were significantly more likely to deliver their babies at <35 weeks of gestation (36% vs. 16%) and to have infants who were small for gestational age (23% vs. 10%).

Conclusions In women with chronic hypertension, the presence of proteinuria early in pregnancy is associated with adverse neonatal outcomes, independent of the development of preeclampsia.