Pediatr Nephrol (2014) 29:2139–2146
DOI 10.1007/s00467-014-2847-y

ORIGINAL ARTICLE

Methylmalonic acidemia: A megamitochondrial disorder affecting the kidney

Zsuzsanna K. Zsengellér · Nika Aljinovic · Lisa A. Teot · Mark Korson · Nancy Rodig · Jennifer L. Sloan · Charles P. Venditti · Gerard T. Berry · Seymour Rosen

Received: 16 December 2013 / Revised: 30 April 2014 / Accepted: 1 May 2014 / Published online: 28 May 2014
© IPNA 2014

Abstract
Background Classical (or isolated) methylmalonic acidemia (MMA) is a heterogeneous inborn error of metabolism most typically caused by mutations in the vitamin B12-dependent enzyme methylmalonyl-CoA mutase (MUT). With the improved survival of individuals with MMA, chronic kidney disease has become recognized as part of the disorder. The precise description of renal pathology in MMA remains uncertain.
Methods Light microscopy, histochemical, and ultrastructural studies were performed on the native kidney obtained from a 19-year-old patient with *mut* MMA who developed end stage renal disease and underwent a combined liver–kidney transplantation.
Results The light microscopy study of the renal parenchyma in the MMA kidney revealed extensive interstitial fibrosis, chronic inflammation, and tubular atrophy. Intact proximal tubules were distinguished by the widespread formation of large, circular, pale mitochondria with diminished cristae. Histochemical preparations showed a reduction of cytochrome c oxidase and NADH activities, and the electron microscopy analysis demonstrated loss of cytochrome c enzyme activity in these enlarged mitochondria.
Conclusions Our results demonstrate that the renal pathology of MMA is characterized by megamitochondria formation in the proximal tubules in concert with electron transport chain dysfunction. Our findings suggest therapies that target mitochondrial function as a treatment for the chronic kidney disease of MMA.

Keywords Methylmalonic acidemia · Methylmalonyl-CoA mutase · Megamitochondria · Cytochrome c oxidase · End stage renal disease · Functional electron microscopy · Vitamin B12

Electronic supplementary material The online version of this article (doi:10.1007/s00467-014-2847-y) contains supplementary material, which is available to authorized users.

Z. K. Zsengellér
Molecular Medicine, Beth Israel Deaconess Medical Center–Harvard Medical School, Boston, MA, USA

Z. K. Zsengellér (✉) · N. Aljinovic · S. Rosen
Pathology Department, Beth Israel Deaconess Medical Center–Harvard Medical School, 330 Brookline Avenue, Boston, MA 02215, USA
e-mail: zzsengel@bidmc.harvard.edu

L. A. Teot · S. Rosen
Pathology Department, Boston Children's Hospital–Harvard Medical School,, Boston, MA, USA

M. Korson
Tufts University School of Medicine, Boston, MA, USA

N. Rodig
Division of Nephrology, Boston Children's Hospital–Harvard Medical School, Boston, MA 02115, USA

J. L. Sloan · C. P. Venditti
Organic Acid Research Section, Genetics and Molecular Biology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA

G. T. Berry
Manton Center for Orphan Disease Research, Boston Children's Hospital–Harvard Medical School, Boston, USA

Introduction

Renal tubular dysfunction with progression into chronic tubulointerstitial nephritis and end stage renal disease is a cardinal manifestation of methylmalonic acidemia (MMA), a common and severe organic acidemia characterized by metabolic instability, multi-systemic complications, and high

mortality [1]. Isolated MMA is primarily caused by mutations in the vitamin B12-dependent, mitochondrial matrix-localized methylmalonyl-CoA mutase (MUT), an enzyme that mediates the entry of carbon skeletons derived from branched-chain amino acids, odd-chained fatty acids, and cholesterol oxidation into the Krebs cycle Fig. 1. MUT deficiency may be complete (*mut*^{0}) or partial (*mut*–) or caused by diminished synthesis of the cofactor 5′-deoxyadenosylcobalamin. The latter forms are associated with *cblA*, *cblB*, or *cblD-variant 2* complementation groups caused by mutations in *MMAA*, *MMAB*, or *MMADHC* and are more likely to be responsive to vitamin B12, a factor associated with an improved prognosis [2–5].

Renal disease and ensuing kidney dysfunction have become an increasingly important clinical manifestation of isolated MMA [5–7]. While the original description of the disorder clearly documented both microscopic pathology and tubular dysfunction in the index cases reported in 1967 [8], chronic kidney disease was not fully appreciated as a disease-related complication until the late 1980s [6], when some severely affected patients began surviving past the first decade of life. Since then, retrospective, survey-based studies [5, 7] as well as case series [9] have confirmed and extended previous observations. The trend that has emerged is that vitamin B12 non-responsive MMA patients, particularly those of the *mut*^{0} enzymatic subtype, frequently develop chronic kidney disease [5, 7], and in one study based on clinical experience, with a median age of onset of 6.5 years [9]. While early disease progression remains difficult to discern, smaller renal length and higher serum methylmalonic acid levels appear to correlate with more severe renal dysfunction [10].

What has remained unexplored is the precise morphological and histopathological characterization of the chronic kidney disease of MMA, which has typically been described as tubulointerstitial nephritis without specific features [11, 12]. The study of methylmalonyl-CoA mutase knockout mice (*Mut*$^{-/-}$) has suggested that megamitochondria formation in selected cell types, such as hepatocytes and renal proximal tubule epithelial cells, may be a cardinal manifestation of the pathology of MMA [13]. The fact that both *Mut*$^{-/-}$ mice [13] as well as rats treated with a toxic vitamin B12 analog, OH-c-lactam, develop a similar hepatic mitochondrial morphological phenotype associated with depressed NADH and cytochrome c oxidase (COX) activity suggests that megamitochondria formation in MMA is associated with a specific pattern of electron transport chain (ETC) dysfunction [14–16]. Whether similar changes occur in selected cell types in patients and if present whether they are also associated with a phenotype of impaired ETC activity have not been fully explored. In this report, light microscopy, ultrastructural and enzymatic studies on the native kidney excised from a 19-year-old patient with vitamin B12 non-responsive *mut* MMA

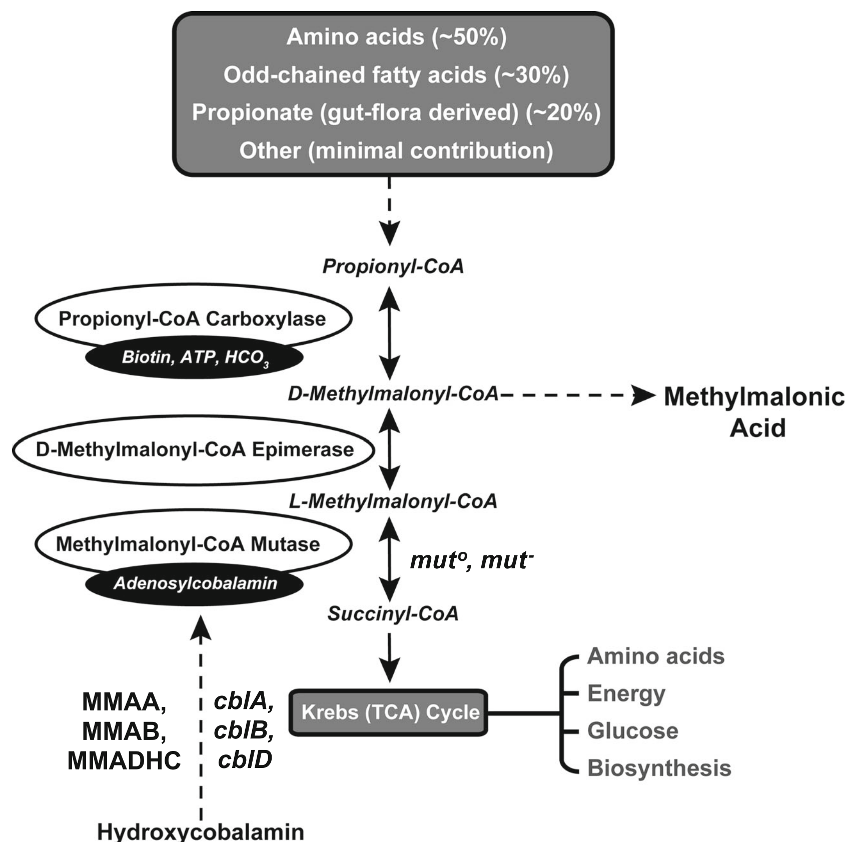

Fig. 1 The intra-mitochondrial metabolism of propionyl-CoA into succinyl-CoA. Isolated methylmalonic acidemia (MMA) results from deficiencies in the conversion of D-methylmalonyl-CoA to succinyl-CoA and can be caused by mutations in methylmalonyl-CoA mutase (*MUT*) as well as defects in the synthesis of adenosylcobalamin (*MMAA*, *MMAB*, *MMADHC*). The complementation groups that correspond to each gene are presented *next* to the corresponding enzyme

who underwent a combined liver–kidney transplantation (LKT) are presented. Using enzyme–histochemistry imaged by light or electron microscopy, we demonstrate diminished COX and NADH oxidase staining as an intrinsic change that characterizes the pathology of the proximal tubular mitochondropathy of MMA.

Methods

Clinical and molecular studies The renal tissue used in these studies was derived from the kidney of a 19-year-old patient who underwent a combined LKT. Unfortunately, the patient passed away a few months after the transplant procedure and no autopsy was obtained.

A liver sample collected at the time of transplantation was used as a source of DNA to determine the sequence of the MUT gene using a previously established method. PCR products were purified and subjected to direct Sanger sequencing of exons 1–12 and 13a and 13b in the MUT gene, and the results were compared to the reference sequence and previously reported mutations.

Histology The kidney was processed for unfixed frozen preparation for enzyme histochemistry, formalin-fixed paraffin embedding for routine histology, or 2 % glutaraldehyde fixed for electron microscopy (EM). A separate preparation was used for functional EM, as described below.

In situ enzyme chemistry–light microscopy After removal, the kidney cortex and medulla was frozen in isopentane cooled in liquid nitrogen. The tissues was cryo-sectioned (thickness 6 μm) and stained for NADH and COX activities, as described previously [17–19].

In situ enzyme chemistry–EM Freshly frozen kidney tissue was cryo-sectioned at 60 μm and fixed in 2.5 % glutaraldehyde in 0.1 M Na-cacodylate buffer (Tousimis Research Corp. Rockville, MD) for 20 min. Sections were washed in several changes of 0.05 M phosphate buffered saline (pH 7.4) for 1.5 h. The sections were treated for enzyme chemistry as described previously [20–23], then processed for EPON embedding using standard protocols. Sections (thickness 0.5 μm) were examined with a JEOL 1011 Transmission Electron Microscope (JEOL Corp., Tokyo, Japan), with a Hamamatsu Orca-HR Digital Camera (Hamamatsu Corp., San Jose, CA), and AMT image capture system (Advanced Microscopy Techniques Corp., Woburn, MA).

Mitochondrial measurements A control kidney and that of the MMA patient were fixed in 2.5 % glutaraldehyde. A tissue block of approximately 5 mm^3 was collected from each kidney, including a portion of the renal cortex and outer medulla for standard processing for EM. The tissue blocks were first examined at low magnification (×3,000) to identify representative proximal and distal tubules. Cells in these tubules were then examined at high magnification (×10,000) to observe mitochondria. To approximate mitochondrial volume, we collected digital images with scale bars. The volume of individual mitochondria in a cell was measured by tracing using NIH ImageJ software (http://rsbweb.nih.gov/ij/). For each tissue sample, we measured approximately 100 mitochondria in a representative area.

Statistics Quantitative results were expressed as mean ± standard error of the mean. Statistical differences between the means were determined using analysis of variance followed by Tukey's post hoc test. A p value of <0.05 was considered to be significant.

Results

The patient had MMA due to a severe MUT deficiency and at 19 years of age underwent a combined LKT. She presented at 5 days of age with coma, metabolic ketoacidosis, and massive hyperammonemia (plasma NH_4>2000 μmol/L) and received hemodialysis. There was no clinical or biochemical response to parenteral vitamin B12, and her condition was managed by adherence to a low-protein diet with L-carnitine supplementation. She had hypotonia, moderate cognitive impairment, and learning difficulties. There were episodes of metabolic decompensation (5 hospitalizations) from the neonatal period until adolescence when she developed progressive renal dysfunction. Thus, while generally stable during her first decade of life, with only occasional metabolic decompensation, by 18 years of age the patient manifested labile hypertension, anemia, and renal failure, and was determined to be a candidate for a combined LKT.

At that time, she was on a low-protein diet, a supplemental amino acid powder devoid of isoleucine, valine, threonine, and methionine, 3,960 mg carnitine per day, 150 meq Bicitra (sodium citrate and citric acid) per day, allopurinol, amlodipine, Renagel (sevelamer hydrochloride), 420 mg valine per day, and 390 mg isoleucine per day. Pre-transplant brain magnetic resonance imaging revealed that cerebral sulci were prominent as were ventricles secondary to mild diffuse parenchymal volume loss. There were moderate atrophic changes to the cerebellum, pons, and mammillary bodies.

Prior to beginning dialysis therapy, the serum methylmalonic acid level was 1,279,162 nmol/L (normal range 73–271 nmol/L); during outpatient visits it was 4,123,296 and 5,846,444 nmol/L, respectively. The plasma total carnitine was 772 μmol/L (normal range 32–84 μmol/L) and free carnitine was 88 μmol/L (normal range 26–60 μmol/

L). The serum methylmalonic acid was 2,126,813 nmol/L in the hours preceding the combined LKT.

Approximately 2 months after initiating chronic hemodialysis, she underwent combined LKT, which included a bilateral nephrectomy. Both kidneys of the patient were small (77 and 59 g, respectively). There were extensive changes of tubular atrophy, fibrosis, and chronic inflammation, all of which were prominent in the subcapsular zone but also involved the medullary rays and adjacent labyrinth Fig. 2a The proximal tubules showed vacuolar changes Fig. 2b. Fine structural studies showed that many of the intact/nonatrophic proximal tubules had a fairly well-maintained brush border, but mitochondrial enlargement with loss of cristae was very prominent Figs. 2c, 3a. The basolateral membranes were reduced. Large vacuoles were also evident, not obviously of mitochondrial origin and perhaps endocytotic in nature. Such changes were not present in the distal tubules Fig. 3c. Indeed, morphometric analysis demonstrated that mitochondrial volume was markedly increased in the kidney proximal tubules in the MMA kidney, compared to control kidney mitochondria Fig. 3d [0.4 ±0.04 (control) vs. 1.7±0.2 μm^2 (MMA); $p<0.005$]. Mitochondrial volume in the distal tubules of the MMA kidney was not changed relative to that in the control kidney, showing that mitochondrial changes were relegated to the tubular proximal segments Fig. 3d.

The activity of COX isoforms was measured using enzyme histochemistry methods and imaged by light microscopy in the kidneys of the MMA patient and those of an anonymous human control (Fig. 4). This assay showed a focal decrease in COX enzyme activity in the tubular epithelium of the renal cortex of the MMA kidney (Fig. 4c, d) compared to that of the control kidney in which COX activity was diffusely intense in the tubules (Fig. 4a, b). Similarly, we found focally decreased NADH enzyme activity in the tubular epithelium of the MMA kidney (Fig. 5c, d), while in control kidney NADH activity was diffusely intense (Fig. 5a, b), thereby demonstrating that more than one component of the ETC is affected/impaired in MMA.

To visualize changes in COX enzymatic activity along the nephron more closely, we applied a technique that combines transmission EM with enzyme histochemistry (Fig. 6). Strong COX enzyme activity was seen in the mitochondria of proximal tubules of the normal (control) kidney (Fig. 6a). In contrast, megamitochondria with disorganized cristae were observed in proximal tubules of the MMA kidney, as well as significantly decreased COX activity (Fig. 6b).

Because cellular biochemical studies were not undertaken and a cell line from the patient was not established, we performed *MUT* sequencing on DNA extracted from the native liver. These studies revealed the presence of two distinct heterozygous *MUT* mutations, one in exon 3, c.654A>C p. Q218H, and another in exon 11, c.1889G>A p. G630E (Electronic Supplemental Material Fig. 1). Both mutations have been previously reported in patients with enzymatically confirmed *mut^o* class MMA and are consistent with the clinical and biochemical phenotype of vitamin B12 non-responsive MMA displayed by the patient [24–27].

Discussion

Chronic kidney disease in MMA is slowly progressive with an incompletely understood natural history. There has been a paucity of nephropathological studies of MMA-associated kidney disease, and the published renal biopsies all show a predominantly tubulointerstitial process that is not reportedly distinctive [11, 12]. While the extent and timing of glomerular dysfunction has been assumed to present years after the initial diagnosis, tubular disease and even a diminution of measured glomerular filtration rate have been documented in MMA

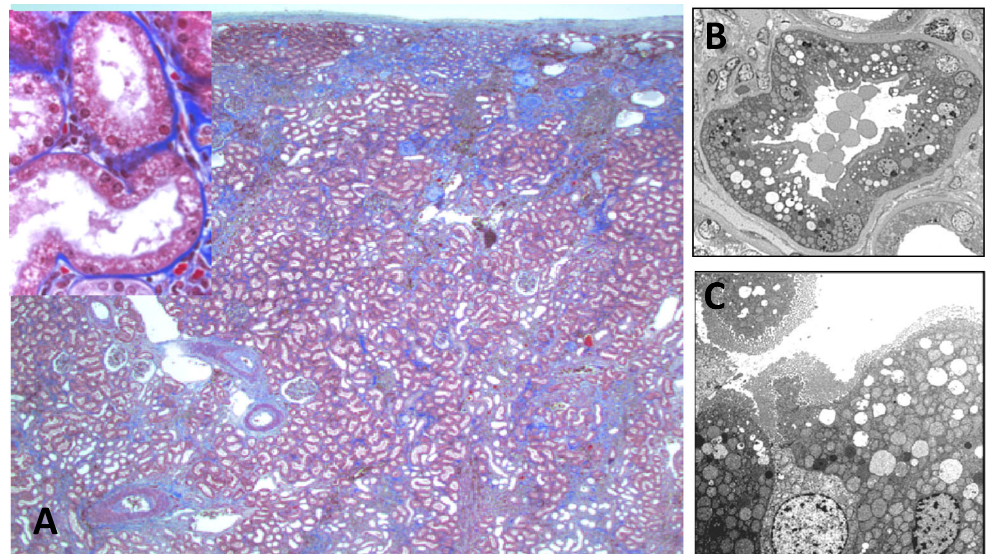

Fig. 2 Native kidney of our patient with methylmalonic acidemia (MMA). **a** Fibrosis is particularly evident in the subcapsular zone; intact parenchyma is hypertrophic. *Inset* High power shows vacuoles in proximal tubular epithelium; Masson Trichrome stain. **b**, **c** Proximal tubule with apical endocytotic vesicles and enlarged mitochondria (**b**), which can be seen better at high power in the electron microscopy (EM) preparation (**c**). Magnification: 10× (**a**), 1,500× (**b**), 3,000× (**c**)

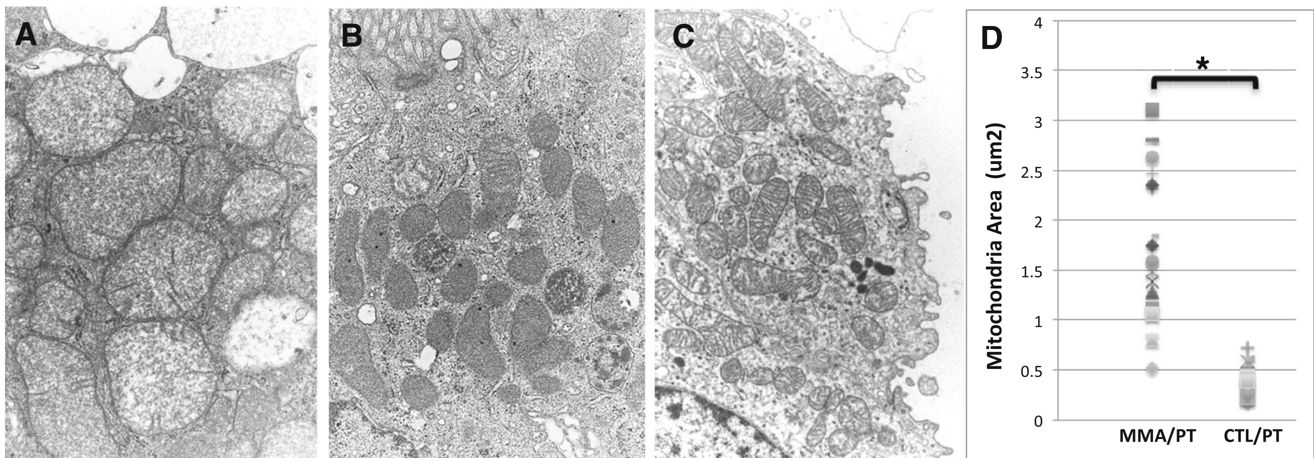

Fig. 3 Mitochondrial measurements in kidney tubules of the methylmalonic acidemia (MMA) kidney. **a**–**c** Megamitochondria can be seen in the MMA kidney (**a**), but are not observed in the proximal tubules (*PT*) of the control kidney (**b**) or in the distal tubules of the kidney from our patient with MMA (**c**). Magnification: 10,000× (**a**), 6,300× (**b**), 10,000× (**c**). **d** Morphometric analysis of mitochondrial volume in the tubular epithelium of the normal and MMA kidney. The mitochondrial areas of the proximal tubular mitochondria (*MMA/PT*) are compared to proximal tubular mitochondria from a control (*CTL/PT*). In each sample, 100 mitochondria were analyzed. *$p<0.001$

patients in early childhood [6]. However, the clinical progression of MMA kidney disease, assessed in parallel with microscopic and ultrastructural analyses, has remained totally unexplored.

Proximal tubular megamitochondria formation as a distinctive feature of pathology of Mut deficiency has been documented in mouse models of MMA [13, 28]. Megamitochondria have been reported to be common in proximal tubules, but under nonspecific conditions, sporadically and as single forms [29]. They occur in greater numbers after acute exposure to nephrotoxins [30, 31] and have been rarely documented in a number of genetic disorders involving the kidney [32, 33]. Perhaps the most common form of mitochondrial enlargement is that involved in ischemia [34], but this change is part of a generalized process associated with loss of global tubular functional and transport capabilities. The ensuing alterations manifest a more pronounced mitochondrial phenotype because of the extensive injury and relative intactness of cellular elements. The mitochondrial changes in the proximal tubular epithelia seen in MMA are comparatively milder, predicting that the ensuing progression of renal disease would be more slowly progressive, as our case clinically demonstrates.

There has long been a suspicion of dysfunctional energy metabolism in MMA, first arising from the study of Hayasaka et al. who showed markedly diminished COX activity in a postmortem liver extract from a single patient with MMA [35]. Indeed, in the livers of MMA mutant mice

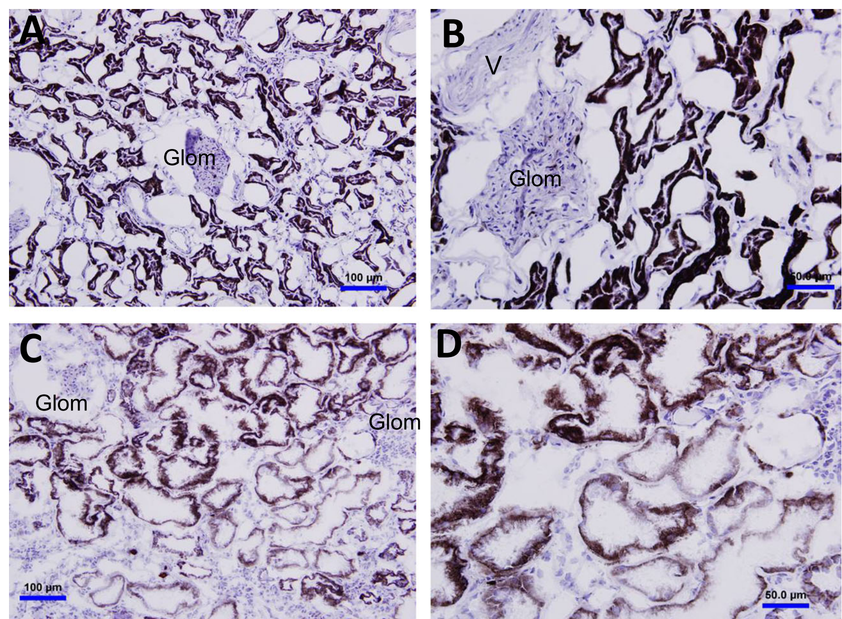

Fig. 4 Cytochrome c oxidase (*COX*) enzyme histochemistry in the renal cortex of methylmalonic acidemia (MMA) kidney and control kidney. **a**, **b** Control kidney, with intense COX enzyme activity homogeneously distributed throughout all tubules (*brown staining* in tubules). **c**, **d**, MMA kidney, with enzyme activity considerably decreased relative to the control kidney, but focally maintained. *Glom* Glomerulus. *Scale bars*: 100 µm (**a**, **c**); 50 µm (**b**, **d**)

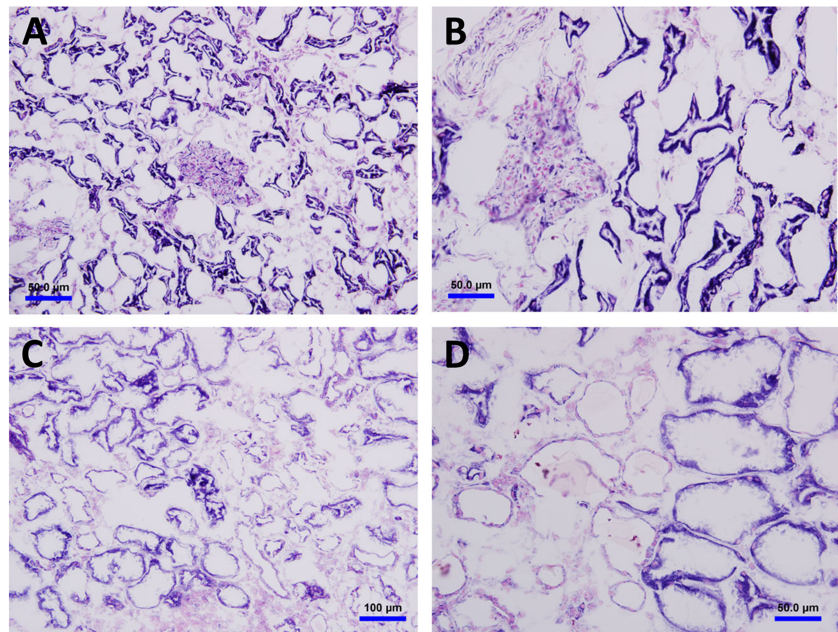

Fig. 5 NADH enzyme histochemistry in the renal cortex of methylmalonic acidemia (MMA) kidney and control kidney. **a**, **b** Control kidney, with intense NADH enzyme activity homogeneously distributed throughout all tubules (*blue staining* in tubules). **c**, **d**, MMA kidney, with enzyme activity considerably decreased in some tubules, but focally maintained in others. *Scale bars*: 100 μm (**a**, **c**); 50 μm (**b**, **d**)

(*Mut*-) and in another single MMA patient, hepatic megamitochondria associated with severely reduced COX activity and mildly depressed NADH oxidase activity (mice only) have been documented [13]. In a related clinical study, de Keyzer et al. examined respiratory chain and tricarboxylic acid cycle enzyme activities in varied tissues in a small series of patients with MMA and the related disorder of propionic acidemia [36]. Renal tissue from a single MMA patient clearly showed the depression of all components of the ETC, but results from pathologic investigations were not presented. This is in contrast to observations from kidney histochemical preparations of *Mut* knockout mice [13] where enzyme activity was found to be present as a mixture of COX-positive and COX-negative tubules, but cumulative tissue levels of respiratory enzymes per se were unchanged. The pattern of mosaic deficiency, with COX and NADH histochemical preparations both showing a reduction of activity in intact epithelium without changes in atrophy, was clearly seen in the kidney of our patient. Our additional demonstration of reduced COX activity evidenced by EM–histochemical study proves that intact megamitochondria have reduced staining—not because of tubular atrophy and ensuing secondary changes but because the intact enlarged mitochondria themselves have reduced activity. This specific observation is the first to directly demonstrate cell autonomous COX deficiency in renal tubular megamitochondria in MMA, further extending observations from prior animal studies. The in situ proximal tubular COX deficiency we describe may therefore represent a specific nephropathological marker temporally associated with kidney disease progression in MMA, the assay of which could expand the utility of renal biopsies

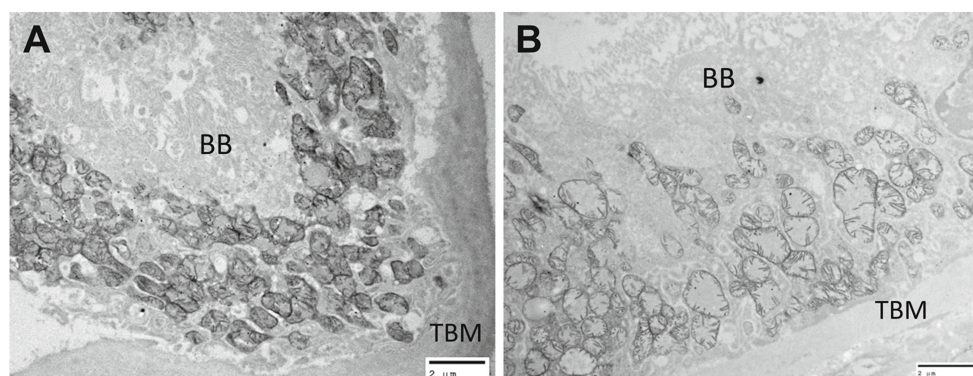

Fig. 6 Ultrastructural analysis (EM + enzyme histochemistry) of mitochondrial COX enzyme activity in proximal tubules of the methylmalonic acidemia (MMA) kidney and control kidney. **a** Renal cortex of the control kidney, with an intact proximal tubular epithelium with preserved mitochondria and intense COX activity throughout all mitochondria. **b** MMA kidney, with loss of enzyme activity and presence of disorganized mitochondria, including megamitochondria, with disrupted cristae and greatly reduced COX activity. *TBM* Tubular basement membrane, *BB* brush border. *Scale bars*: 2 μm

to gauge the extent of MMA renal pathology independent of tubulointerstitial inflammatory changes.

Acknowledgments We thank Ms. Lena Ellezian for cryosectioning and electron microscopy processing at Department of Pathology at BIDMC. We also thank Ms. Andrea Calhoun at the Electron Microscopy Core Laboratory of BIDMC for her assistance with the COX–electron microscopy study.

References

1. Manoli I, Venditti CP (2005, update 2010). Methylmalonic acidemia. In: Pagon RA, Bird TD, Dolan CR et al (eds) GeneReviews™. University of Washington, Seattle. Available at: 1993 http://www.ncbi.nlm. nih.gov/books/NBK1231/
2. Matsui SM, Mahoney MJ, Rosenberg LE (1983) The natural history of the inherited methylmalonic acidemias. N Engl J Med 308: 857–861
3. Nicolaides P, Leonard J, Surtees R (1998) Neurological outcome of methylmalonic acidaemia. Arch Dis Child 78: 1508–512
4. van der Meer SB, Poggi F, Spada M, Bonnefont JP, Ogier H, Hubert P, Depondt E, Rapoport D, Rabier D, Charpentier C, ParvyP BJ, Kamoun P, Saudubray JM (1994) Clinical outcome of long-term management of patients with vitamin B12-unresponsive methylmalonic acidemia. J Pediatr 125(6 Pt 1):903–908
5. Horster F, Baumgartner MR, Viardot C, Suormala T, Burgard P, Fowler B, Hoffmann GF, Garbade SF, Kölker S, Baumgartner ER (2007) Long-term outcome in methylmalonic acidurias is influenced by the underlying defect (mut0, mut-, cblA, cblB). Pediatr Res 2: 225–230
6. Walter JH, Michalski A, Wilson WM, Leonard JV, Barratt TM, Dillon MJ (1989) Chronic renal failure in methylmalonic acidaemia. Eur J Pediatr 148:344–348
7. Baumgarter ER, Viardot C (1995) Long-term follow-up of 77 patients with isolated methylmalonic acidaemia. J Inherit Metab Dis 18: 138–142
8. Oberholzer VG, Levin B, Burgess EA, Young WF (1967) Methylmalonic aciduria. An inborn error of metabolism leading to chronic metabolic acidosis. Arch Dis Child 42:492–504
9. Cosson MA, Benoist JF, Touati G, Déchaux M, Royer N, Grandin L, Jais JP, Boddaert N, Barbier V, Desguerre I, Campeau PM, Rabier D, Valayannopoulos V, Niaudet P, de Lonlay P (2009) Long-term outcome in methylmalonic aciduria: a series of 30 French patients. Mol Genet Metab 97:172–178
10. Kruszka PS, Manoli I, Sloan JL, Kopp JB, Venditti CP (2013) Renal growth in isolated methylmalonic acidemia. Genet Med 15(12):990–996. doi:10.1038/gim.2013.42
11. Molteni KH, Oberley TD, Wolff JA, Friedman AL (1991) Progressive renal insufficiency in methylmalonic acidemia. Pediatr Nephrol 5:323–326
12. Rutledge SL, Geraghty M, Mroczek E, Rosenblatt D, Kohout E (1993) Tubulointerstitial nephritis in methylmalonic acidemia. Pediatr Nephrol 7:81–82
13. Chandler RJ, Zerfas PM, Shanske S, Sloan J, Hoffmann V, DiMauro S, Venditti CP (2009) Mitochondrial dysfunction in *mut* methylmalonic acidemia. FASEB J 23:1252–61
14. Tandler B, Krahenbuhl S, Brass EP (1991) Unusual mitochondria in the hepatocytes of rats treated with a vitamin B12 analogue. Anat Rec 231:1–6
15. Stabler SP, Brass EP, Marcell PD, Allen RH (1991) Inhibition of cobalamin-dependent enzymes by cobalamin analogues in rats. J Clin Invest 87:1422–1430
16. Krahenbuhl S, Chang M, Brass EP, Hoppel CL (1991) Decreased activities of ubiquinol:ferricytochrome c oxidoreductase (complex III) and ferrocytochrome c:oxygen oxidoreductase (complex IV) in liver mitochondria from rats with hydroxycobalamin [c-lactam]-induced methylmalonic aciduria. J Biol Chem 266: 20998–21003
17. Hershey SJ, Simon TW, Baste C (1975) Histochemical localization of cytochrome oxidase in gastric mucosa. J Histochem Cytochem 4: 271–282
18. Dubowitz V (1985) Muscle biopsy: A practical approach, 2nd edn. Lavenham Press, Lavenham, Suffolk
19. Lebrecht D, Setzer B, Rohrbach R, Walker UA (2004) Mitochondrial DNA and its respiratory chain products are defective in doxorubicin nephrosis. Nephrol Dial Transplant 19:329–336
20. Seligman AM, Karnovsky MJ, Wasserkrug HL, Hanker JS (1968) Nondroplet ultrastructural demonstration of cytochrome oxidase activity with a polymerizing osmiophilic reagent, diaminobenzidine (DAB). J Cell Biol 1:1–14
21. Anderson WA, Bara G, Seligman AM (1975) The ultrastructural localization of cytochrome oxidase via cytochrome. J Histochem Cytochem 1:13–20
22. Bertoni-Freddari C, Fattoretti P, Casoli T, Di Stefano G, Solazzi M, Gracciotti N, Pompei P (2001) Mapping of mitochondrial metabolic competence by cytochrome oxidase and succinic dehydrogenase cytochemistry. J Histochem Cytochem 9:1191–1192
23. Zsengellér ZK, Ellezian L, Brown D, Horváth B, Mukhopadhyay P, Kalyanaraman B, Parikh SM, Karumanchi SA, Stillman IE, Pacher P (2012) Cisplatin nephrotoxicity involves mitochondrial injury with impaired tubular mitochondrial enzyme activity. J Histochem Cytochem 7:521–529
24. Worgan LC, Niles K, Tirone JC, Hofmann A, Verner A, Sammak A, Kucic T, Lepage P, Rosenblatt DS (2006) Spectrum of mutations in *mut* methylmalonic acidemia and identification of a common Hispanic mutation and haplotype. Hum Mutat 1:31–43
25. Lempp TJ, Suormala T, Siegenthaler R, Baumgartner ER, Fowler B, Steinmann B, Baumgartner MR (2007) Mutation and biochemical analysis of 19 probands with mut0 and 13 with mut- methylmalonic aciduria: identification of seven novel mutations. Mol Genet Metab 3: 284–290
26. Fuchshuber A, Mucha B, Baumgartner ER, Vollmer M, Hildebrandt F (2000) mut0 methylmalonic acidemia: eleven novel mutations of the methylmalonyl CoA mutase including a deletion-insertion mutation. Hum Mutat 2:179
27. Crane AM, Ledley FD (1994) Clustering of mutations in methylmalonyl CoA mutase associated with mut- methylmalonic acidemia. Am J Hum Genet 1:42–50
28. Manoli I, Sysola JR, Lib L, Houillier P, Garone C, Wang C, Zerfas PM, Cusmano-Ozog K, Young S, Trived Niraj S, Cheng J, Sloan J, Chandler R, Abu-Asab M, Tsokos M, Elkahloun A, Rosen S, Enns G, Berry G, Hoffmann V, DiMauro S, Schnermann J, Venditti CP (2013) Targeting proximal tubule mitochondrial dysfunction attenuates the renal disease of methylmalonic acidemia. Proc Natl Acad Sci USA 110:13552–13557
29. Suzuki T, Furusato M, Takasaki S, Ishikawa E (1975) Giant mitochondria in the epithelial cells of the proximal convoluted tubules of diseased human kidneys. Lab Invest 33:578–590
30. Mihatsch MJ, Ryffel B, Hermle M, Brunner FP, Thiel G (1986) Morphology of cyclosporine nephrotoxicity in the rat. Clin Nephrol 25[Suppl 1]:S2–8
31. Herlitz LC, Mohan S, Stokes MB, Radhakrishnan J, D'Agati VD, Markowitz GS (2010) Tenofovir nephrotoxicity: acute tubular necrosis with distinctive clinical, pathological, and mitochondrial abnormalities. Kidney Int 78:1171–1177
32. Kitano A, Nishiyama S, Miike T, Hattori S, Ohtani Y, Matsuda I (1986) Mitochondrial cytopathy with lactic acidosis, carnitine deficiency and DeToni-Fanconi-Debré syndrome. Brain Dev 8:289–295

33. Davis RL, Sue CM (2011) The genetics of mitochondrial disease. Semin Neurol 31:519–530
34. Wakabayashi T (2002) Megamitochondria formation—physiology and pathology. J Cell Mol Med 6:497–538
35. Hayasaka K, Metoki K, Satoh T, Narisawa K, Tada K, Kawakami T, Matsuo N, Aoki T (1982) Comparison of cytosolic and mitochondrial enzyme alterations in the livers of propionic or methylmalonic acidemia: a reduction of cytochrome oxidase activity. Tohoku J Exp Med 137:329–334
36. de Keyzer Y, Valayannopoulos V, Benoist JF, Batteux F, Lacaille F, Hubert L, Chrétien D, Chadefeaux-Vekemans B, Niaudet P, Touati G, Munnich A, de Lonlay P (2009) Multiple OXPHOS deficiency in the liver, kidney, heart, and skeletal muscle of patients with methylmalonic aciduria and propionic aciduria. Pediatr Res 66:91–95