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Renal involvement in a patient with cobalamin A type (cblA) methylmalonic aciduria: A 42-year follow-up

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Abstract: Chronic renal failure is a well-known long-term complication of methylmalonic aciduria (MMA-uria), occurring even under apparently optimal metabolic management. The onset of renal dysfunction seems to be dependent on the type of defect and vitamin B12-responsiveness. We report on a patient with a vitamin B12-responsive cobalamin A type (cblA) MMA-uria caused by a homozygous stop mutation (p.R145X) in the cobalamin A gene (MMAA). She was diagnosed with chronic kidney disease (CKD) stage III at the age of 12years. Following re-evaluation, the patient received vitamin B12 (hydroxocobalamin) treatment, resulting in a significant decrease in the concentration of methylmalonic acid (MMA) in urine and plasma. Until age 29years glomerular filtration rate remained stable probably due to hydroxocobalamin treatment slowing down progression to end-stage renal failure. Kidney biopsies showed non-specific manifestations of chronic interstitial inflammation. The patient received a renal transplant at age 35years. Under continuous treatment with hydroxocobalamin there is no evidence of kidney damage due to MMA-uria until the last follow-up 6years after transplantation. This case report illustrates (i) a long-term follow-up of a patient with MMA-uria due to cblA deficiency, (ii) the involvement of the kidney as a target organ and (iii) the importance of early and adequate vitamin B12 substitution in responsive patients. Further investigation will be necessary to prove the protective effect of hydroxocobalamin in the kidney in vitamin B12-responsive patients.

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Chronic renal failure is a well-known long-term complication of methylmalonic aciduria (MMA-uria), occurring even under apparently optimal metabolic management. The onset of renal dysfunction seems to be dependent on the type of defect and vitamin B_{12}-responsiveness. We report on a patient with a vitamin B_{12}-responsive cobalamin A type (cblA) MMA-uria caused by a homozygous stop mutation (p.R145X) in the cobalamin A gene (MMAA). She was diagnosed with chronic kidney disease (CKD) stage III at the age of 12 years. Following re-evaluation, the patient received vitamin B_{12} (hydroxocobalamin) treatment, resulting in a significant decrease in the concentration of methylmalonic acid (MMA) in urine and plasma. Until age 29 years glomerular filtration rate remained stable probably due to hydroxocobalamin treatment slowing down progression to end-stage renal failure. Kidney biopsies showed non-specific manifestations of chronic interstitial inflammation. The patient received a renal transplant at age 35 years. Under continuous treatment with hydroxocobalamin there is no evidence of kidney damage due to MMA-uria until the last follow-up 6 years after transplantation.

This case report illustrates (i) a long-term follow-up of a patient with MMA-uria due to cblA deficiency, (ii) the involvement of the kidney as a target organ and (iii) the importance of early and adequate vitamin B_{12} substitution in responsive patients. Further investigation will be necessary to prove the protective effect of hydroxocobalamin in the kidney in vitamin B_{12}-responsive patients.

1. Introduction

Methylmalonic aciduria (MMA-uria), first described by the group of Oberholzer et al. in 1967 [1], belongs to the group of organic acidurias. It is biochemically characterized by the accumulation of specific organic acids such as methylmalonic acid (MMA), propionate (PA), 3-OH-propionic acid (3-OH-PA) and 2-methylcitric acid (MC). The disease is caused either by mutations (mut^0, mut^-) in the enzyme methylmalonyl-CoA mutase (MCM) which is involved in the breakdown of branched chain amino acids, odd-numbered chain fatty acids, cholesterol side chains and other metabolites or by mutations in the genes MMAA (cblA), MMAB (cblB) and MMADHC (cblD-variant2) encoding proteins important for the synthesis of its cofactor adenosylcobalamin (AdoCbl) [2]. According to the type of mutation and resulting residual enzymatic activity, there is a wide clinical spectrum of the disease. Typically, patients manifest with acute metabolic crisis during the first days of life or in catabolic states due to infectious diseases or prolonged fasting. The metabolic crises are characterized by lactic acidosis, hyperketonaemia, hypo- or hyperglycaemia and hyperammonaemia and may result in metabolic encephalopathy and multi-organ failure. Complications include failure to thrive, developmental delay, mental retardation, muscular hypotonia, metabolic stroke like episodes with basal ganglia damage and renal failure [3].

The pathophysiology of chronic kidney disease (CKD) in MMA-uria is poorly understood. There is evidence that the severity of the defect and therefore the concentration of MMA might play an important role [3]. The nature of the disease in the kidney is unclear for the lack of specific biomarkers and investigations of kidney biopsies at an early stage of the disease. Careful clinical descriptions combined with detailed studies of kidney biopsies are necessary to yield insights into the origin of kidney destruction. Here we present the 42-year history of renal involvement as well as analysis of several kidney biopsies in a female patient with vitamin B_{12}-responsive MMA-uria due to a homozygous stop mutation in the MMAA gene (cblA).

2. Case report

The patient was born after uneventful pregnancy as the first child to a non-consanguineous Caucasian couple. She presented at 3 days of life with icterus (bilirubin 340 μmol/l), muscular hypotonia, vomiting, weight loss and lethargy. There was no acidosis present (plasma bicarbonate 25.4 mmol/l) and blood ammonia concentration was normal for a newborn (88 μmol/l). The first profile of amino acids in plasma and urine revealed elevated concentrations of lysine and hyperlysinemia was ruled out. Under suspicion of a metabolic defect the child was put on a low protein diet (1.4 g protein/kg/d) and improved remarkably. The follow-up investigation of the amino acid profile showed hyperglycinemia and hyperglycinuria. Accidental protein intake of 2 g/kg/d at 4 month of age led to a metabolic derailment which could be easily controlled by i.v. glucose application. At age 8 months the urine was screened for organic acids and high concentrations of MMA were detected in urine (4.3 mmol/d) and plasma (255 μmol/l) establishing the diagnosis of MMA-uria. Incorporation of label from [^{14}C]propionate into cell proteins in cultured fibroblasts was severely deficient but showed a clear response to supplementation of the culture medium with high concentrations of hydroxocobalamin. A cblA defect was confirmed by somatic complementation and later by molecular genetic investigation which revealed a homozygous mutation in exon 2 of the MMAA gene (c.433C NT) predicting an amino acid change from arginine to a stop codon at position 145 (p.R145X). Vitamin B_{12} concentration was low (225 pmol/l) but within the normal range (220–730 pmol/l). Intramuscular injections of hydroxocobalamin for 5 days did not change the concentration of excreted MMA. Accordingly, no treatment was started initially. The patient was kept on a low protein diet (1.4 g/kg/d) and showed normal growth and psychomotor development (IQ 98 at age 4 years by J. Kramer's intelligence test) [Fig. 1].

At age 12 years, the patient showed impaired growth and CKD stage III was discovered (glomerular filtration rate: 45 ml/min/1.73 m^2, obtained by 24 h urine collection, [Fig. 2]). Until this age, kidney function had not been documented so that the onset of CKD remains obscure. In addition, hyperuricemia (up to 778 μmol/l) due to reduced excretion of uric acid (19 μmol/kg/d, normal range 91 ± 50) was identified and subsequently treated with a xanthine oxidase inhibitor (Allopurinol®). Generalized signs of proximal tubulopathy like that seen in Fanconi syndrome described by D'Angio et al. in a patient with MMA-uria [4] were not found. Kidney size determined by ultrasound was within the normal range. According to a previous publication [5] the responsiveness of this patient to hydroxocobalamin was re-evaluated and, this time, found to be positive. Treatment with hydroxocobalamin was re-introduced using a dosage of 2 × 10 mg/d p.o. from 1983 to 1995 and 1 × 10 mg/7d i.m. from 1995 to 2006. Cofactor responsiveness was documented by impressive catch-up growth [Fig. 1] as well as long-term decline in urinary MMA excretion [Fig. 2], with mean MMA excretion decreasing from 3212 μmol/mmol creatinine before administration to 719 μmol/mmol creatinine (oral treatment) and further to 422 μmol/mmol creatinine (i.m. administration). Treatment included a low protein diet as well as carnitine (3 × 1 g/d) and bicarbonate. Neurodevelopment remained normal (IQ 97 at age 22, by Hamburg-Wechsler-Intelligenztest für Erwachsene, (HAWIE-R)).

Total kidney volume detected by ultrasound decreased during the following years and at 16 years of age it was below two standard deviations compared to healthy peers. From 1988 she had arterial hypertension and in 1989 (age 18), at CKD stage III (GFR 54 ml/min/m^2, determined by inulin clearance), a renal biopsy was performed [Fig. 2, nr. 1, Fig. 3]. The main findings were areas with advanced tubular atrophy and interstitial fibrosis which were interspersed with mononuclear infiltrates. Immunohistochemistry and electron microscopy (EM) ruled out a glomerulonephritis or a hereditary glomerulopathy. Unfortunately, the material was not sufficient to examine the tubulointerstitium by EM in respect to mitochondrial pathologies as seen in the proximal tubules of a Mut knock-out mouse model [6].

From 1996 (age 25), renal function deteriorated. Another biopsy was performed in 1999 (age 28), [Fig. 2, nr. 2] showing a similar pattern as previously reported. However, additionally severe arteriolosclerosis was detected most probably due to arterial hypertension. One year later renal replacement therapy had to be started and the patient was dependent on hemodialysis the following 6 years [Fig. 2].

In 2006 (age 35) the patient underwent kidney transplantation from a deceased donor. After the transplantation she remained on a low protein diet as well as supplementation with hydroxocobalamin (1 × 4 mg/14d i.m.) and carnitine (3 × 1 g/d). Immunosuppressive regimen consisted of tacrolimus (Prograf®, Astellas), mycophenolate-sodium (Myfortic®, Novartis) and prednisone. Prednisone was tapered and stopped three months after transplantation. The estimated glomerular filtration rate (eGFR) according to Cockroft Gault was 90 ml/min/1.73 m^2. Plasma MMA concentration decreased from 304 μmol/l to a baseline of 59 μmol/l [Fig. 2]. Graft function remained stable and surveillance biopsies [Fig. 2, nr. 3, 4] were performed according to the transplant protocol three and six months after transplantation revealing borderline and moderate tubulitis, respectively, both consistent with interstitial rejection. The latter was treated with steroid pulses. In the second year after transplantation (eGFR 68 ml/min/1.73 m^2) a third renal biopsy [Fig. 2, nr. 5] was performed. Besides minor arteriolosclerosis the morphology was well preserved and no further abnormalities were reported. Blood pressure was well controlled under antihypertensive treatment. At the last follow-up, six years after transplantation, serum creatinine concentration was 95 μmol/l, the eGFR was 70 ml/min/1.73 m^2 and there was neither glomerular nor tubular proteinuria detectable.

3. Discussion

We present a long-term follow-up of a patient with vitamin B_{12}-responsive cblA type MMA-uria due to the STOP mutation p.R145X, which is the most common allele found in patients with CblA defects (representing 43% of pathogenic alleles) [7]. The case illustrates progressive loss of kidney function finally leading to end stage kidney failure in a patient with a rather mild form of MMA-uria, in whom development and growth are normal and severe metabolic decompensations are lacking. It also underscores the importance of standardized testing for cofactor responsiveness and subsequent treatment with hydroxocobalamin in such patients. Our data clearly suggest improved metabolic control on i.m. application vs. oral supplementation. Given the risk of these patients for severe long-term complications (e.g. end stage renal disease and optic nerve atrophy) it is important to study the effects of more aggressive management [8].

Patients with isolated MMA-uria, even mildly affected, are thought to be at risk of developing CKD [3]. High concentrations of MMA in urine are a known risk factor for the development of CKD in those patients [3]. The administration of vitamin B_{12} in our patient resulted in a significant decrease in urinary MMA excretion [Fig. 2]. Partial correction of the biochemical phenotype has slowed the progression from CKD III to end stage renal disease. Only after 17 years of vitamin B_{12} supplementation, the patient necessitated hemodialysis. Nevertheless, these results should be interpreted with caution, since the urinary excretion of MMA and other putatively toxic metabolites of alternative propionate oxidation

2 to 20 years: Girls
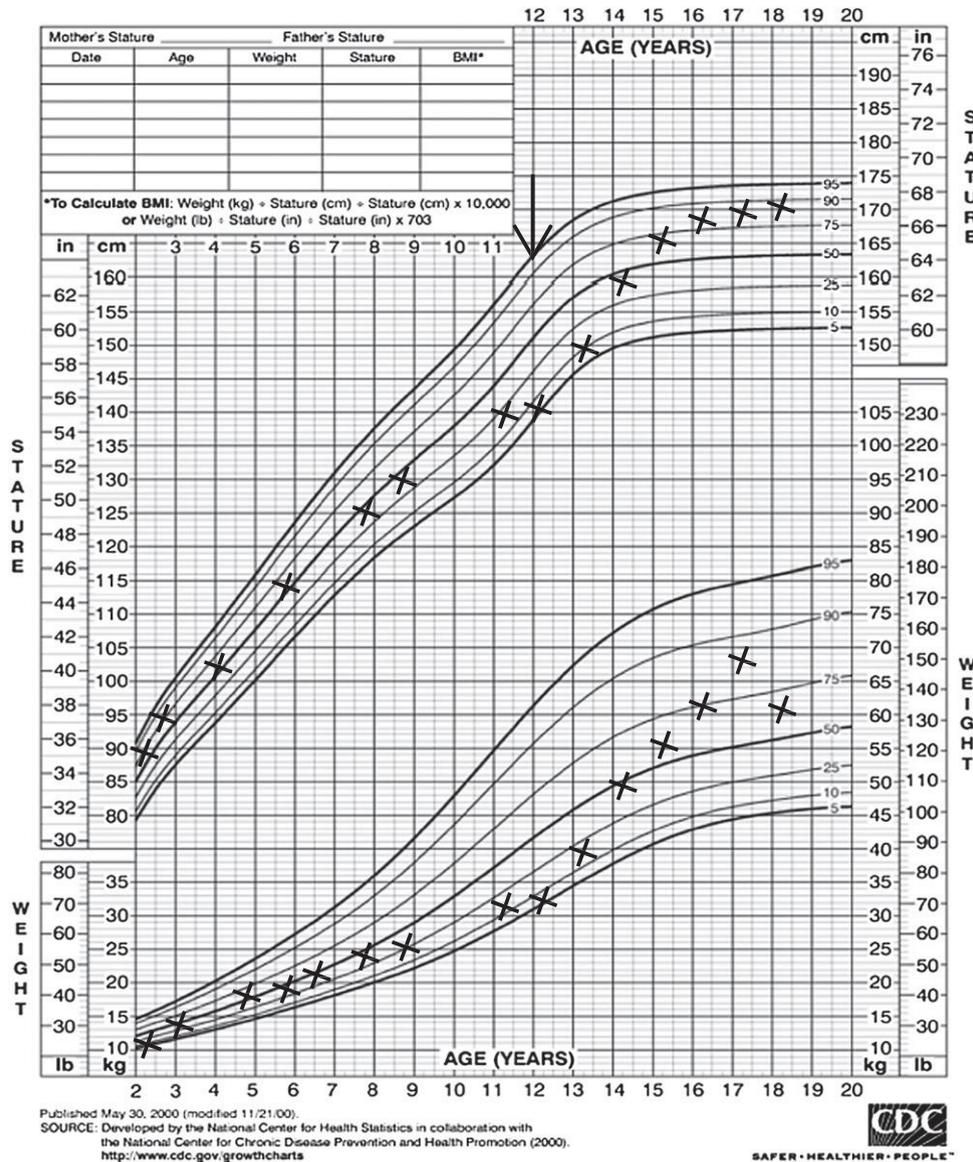**Stature-for-age and Weight-for-age percentiles**

NAME _____

RECORD # _____

Fig. 1. Summary of physical growth. The percentiles show stature and weight-for-age. CKD was diagnosed because of a decline in growth at 12 years of age as indicated by arrowhead.

(e.g. 2-methylcitrate) diminishes with decreasing kidney function and thus accumulating toxic compounds could foster the naturally occurring disease progression despite hydroxocobalamin supplementation. Unfortunately, only very few plasma MMA levels are available during the period before end stage renal disease. The drop of mean plasma as well as urinary MMA concentrations after renal transplantation [Fig. 2] is most likely due to the transplant, as the kidney is said to contribute to at least 18% of activity normally provided by the liver [9,10].

Several mechanisms may explain the pathophysiology of cellular damage in MMA. First, disturbances of the tricarboxylic acid cycle and the respiratory chain as well as disturbances in the glutathione and dicarboxylate transport are thought to be involved in the pathomechanism [11]. Secondary to mitochondrial dysfunction, oxidative stress and disturbances in mitochondrial DNA equilibrium might occur [12–14]. Second, accumulating dicarboxylic acids may be toxic, in particular in the brain. According to the "trapping hypothesis" impaired transport of dicarboxylic acids either at the blood-brain barrier or between astrocytes and neurons as well as de novo synthesis of dicarboxylic acids in brain is causing brain damage [13,14]. Sauer et al.

[11,15] suspect a similar pathomechanism of impaired function of dicarboxylic acid transporters and accumulating mitochondrial toxins to be responsible for kidney damage in MMA-uria.

The performed native kidney biopsies showed areas with remarkable tubular atrophy and interstitial fibrosis interspersed with mononuclear infiltrates without other abnormal findings which might reflect the influence of MMA-uria in the kidney [Fig. 3]. These observations fit with the description of kidney biopsies taken from other patients with MMA-uria due to mut⁻ and cblA deficiency [10,16]. Similarly, the graft biopsies did not show any striking abnormalities which might be a hint at kidney damage caused by MMA-uria. This finding matches with the group of Lubrano et al. [17] who reported the case of a girl receiving a kidney transplant at the age of 17 years because of MMA-uria due to a cblA defect. In a 16.5 year follow-up study renal function remained stable and re-biopsy did not show any striking abnormalities, even during pregnancy [18]. In contrast to our case, this patient is on unrestricted diet and has not been on hydroxocobalamin treatment [18, 19].

Because of the small number of organ transplantations in a phenotypically highly variable patients' population a clear recommendation
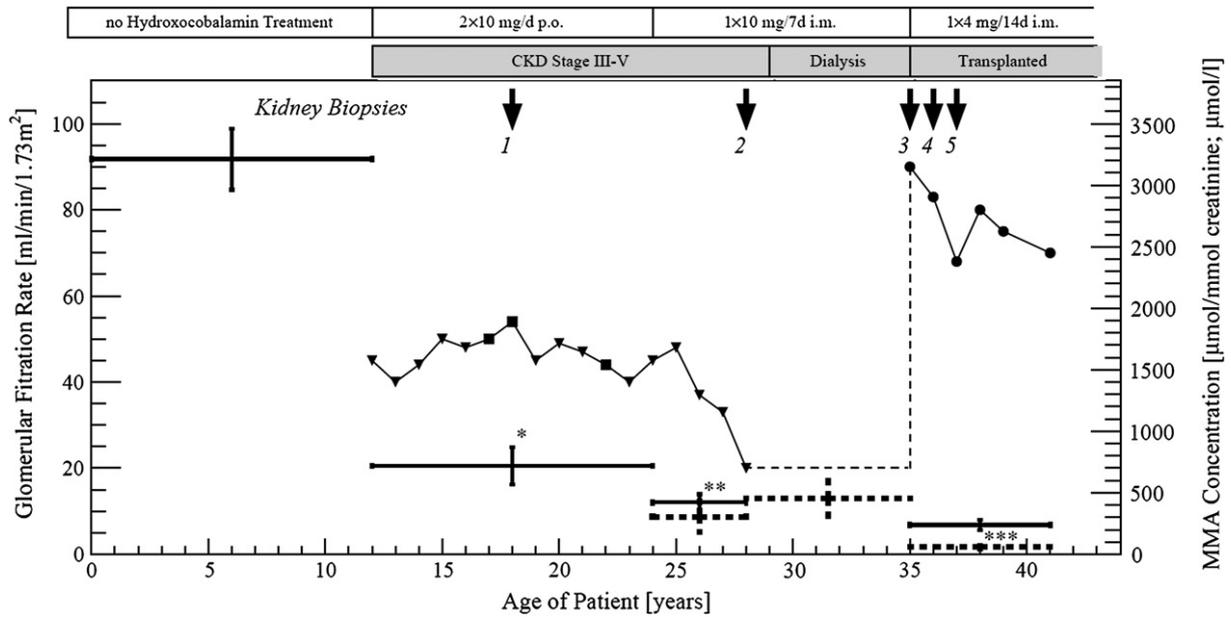
Fig. 2. Course of glomerular filtration rate (GFR) and concentration of MMA in an individual with cblA MMA-uria in dependence of hydroxocobalamin (OHCbl) treatment. Values for GFR indicated as triangles were obtained as mean, each single value obtained from a 24 h urine collection (n = 5 (1983), n = 6 (1984), n = 8 (1985), n = 5 (1986), n = 5 (1987), n = 8 (1990), n = 7 (1991), n = 3 (1992), n = 4 (1994), n = 10 (1995), n = 5 (1996), n = 7 (1997), n = 4 (1998), n = 3 (1999)). GFR values indicated as squares were measured by inulin clearance, values indicated as circles were obtained by Cockroft Gault estimation. Urinary MMA concentrations [μmol/mmol creatinine] are depicted as a black line and plasma MMA concentrations [μmol/l] as a dotted line. Error bars are represented by vertical lines. Kidney biopsies are indicated with arrowheads and numbered. Boxes show 4 periods of treatment with hydroxocobalamin (n = number of independently performed measurements): No hydroxocobalamin treatment (n = 47), 2 × 10 mg/d p.o. (n = 21), 1 × 10 mg/7d i.m. (urine: n = 42, plasma: n = 6), post transplantation 1 × 4 mg/14d i.m. (urine: n = 32, plasma: n = 28). The significance has been determined by one-way ANOVA (*, **P b 0.05) for MMA concentration in urine and (***P b 0.05) for MMA concentration in plasma and is referring to the previous value.

for organ transplantation in MMA-urias cannot be made. Thirty-one patients with MMA-uria with kidney (n = 10), liver (n = 15) or combined liver/kidney transplantation (n = 6) have been reported so far [10, 16, 20–24] and most of them harbor a mut[0] (n = 16) defect. Some patients underwent episodes of severe metabolic decompensation, metabolic stroke or death [25] even after combined liver and kidney transplantation clearly demonstrating that severe complications

may still occur. Nevertheless, the kidney seems to play an important role for metabolic stability and correction of MCM activity in the patients as kidney or combined kidney/liver transplantation seems to lower MMA concentration more efficiently and provides more metabolic stability than single liver transplantation [10, 17, 24, 26]. This case report as well as the findings of other groups [17, 24] in even more severe forms of MMA-uria suggest that elective kidney transplantation
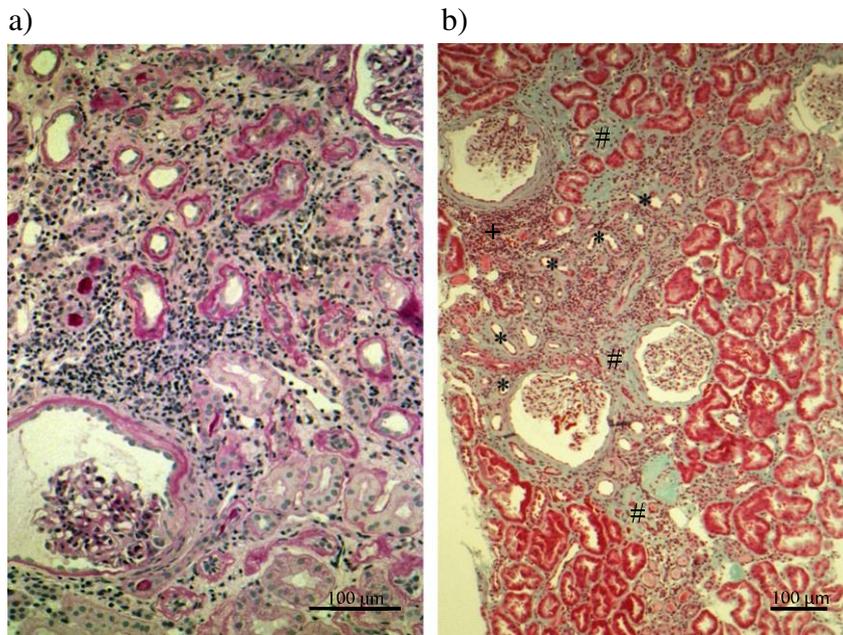

Fig. 3. Native kidney biopsy (1989, age 18 years) showing dense inflammatory interstitial infiltrate (+), areas of tubular atrophy (*) and interstitial fibrosis (#). Enrichment of protein casts close to the medulla–cortex region was reported as well as large areas with completely inconspicuously looking tubules (data not shown in the pictures). a) Periodic acid Schiff stain (PAS), LM, 200×; b) Masson's Trichrome Stain (TRI), LM, 200×.

may be a form of "cell-therapy" and regarded as an alternative and safer strategy than liver or combined liver-kidney transplantation for it seems to restore sufficient enzyme activity and improves quality of life in patients.

Several cases of hyperuricemia in MMA-uria have been reported [10, 27] similar to the finding in our patient. As suggested by the reduced excretion of uric acid, one could speculate that the characteristically accumulating organic acids – and MMA in particular – may induce tubular damage and inhibit the tubular secretion of uric acid. Uric acid is known to induce gout nephropathy via chronic interstitial inflammation [28] and might be an additional toxic factor in the development of CKD in MMA-uria.

In conclusion this case report illustrates that CKD is a long term complication even in patients with mild defects leading to almost no metabolic derailment. High concentrations of MMA are suspected to be nephrotoxic and hydroxocobalamin can lower MMA concentration in responsive patients. Responsiveness to hydroxocobalamin treatment should therefore be carefully evaluated and responsive patients should be adequately treated by parenteral administration since this may slow down the progress of CKD. Elective kidney transplantation seems to be a valid option in selected patients. To date, no evidence of recurrence of MMA-specific changes in the transplanted kidney has been evidenced; however, it has to be kept in mind that it is neither curative nor protective against metabolic stroke.