Clinical and molecular aspects of distal renal tubular acidosis in children

Martine TP Besouw[1], Marc Bienias[2], Patrick Walsh[3], Robert Kleta[1,4], William G van't Hoff[1], Emma Ashton[4], Lucy Jenkins,[4] Detlef Bockenhauer[1,5]

[1] Department of Pediatric Nephrology, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK

[2] Department of Pediatrics, Medical Faculty Carl Gustav Carus, Technical University Dresden, Germany

[3] Great North Children's Hospital, Newcastle upon Tyne NHS Foundation Trust, Newcastle, UK

[4] North East Thames Regional Genetics Service Laboratories, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK

[5] Centre for Nephrology, University College London Institute of Child Health, London, UK

Corresponding author:

Prof. Dr. D. Bockenhauer

Department of Pediatric Nephrology

Great Ormond Street Hospital NHS Trust

Great Ormond Street

London WC1N 3JH

Tel: +44 20 7405 9200

detlef.bockenhauer@gosh.nhs.uk

Abstract

Background: Distal renal tubular acidosis (dRTA) is characterized by hyperchloremic metabolic acidosis, hypokalemia, hypercalciuria and nephrocalcinosis. It is due to reduced urinary acidification by the α-intercalated cells in the collecting duct and can be caused by mutations in *ATP6V1B1*, *ATP6V0A4*, or *SLC4A1*. Treatment with alkali is the mainstay of therapy.

Patients and methods: This study presents clinical data on long-term follow-up of children with dRTA in a single centre, including genetic analysis.

Results: Twenty-four children were included. Genetic diagnosis was confirmed in 19 patients: 6 had mutations in *ATP6V1B1*, 10 in *ATP6V0A4* and 3 in *SLC4A1*, 5 had no molecular diagnosis. There were 5 novel mutations (2 in *ATP6V1B1* and 3 in *ATP6V0A4*). Two thirds of patients presented with features of proximal tubular dysfunction, leading to an erroneous diagnosis of renal Fanconi syndrome. The proximal tubulopathy disappeared after resolution of acidosis, indicating the importance of following proximal tubular function to establish the correct diagnosis. Growth retardation with a height below -2 SDS was found in 10 patients at presentation, but remained present in only 3 once established on alkali treatment. Sensorineural hearing loss was found in 5/6 patients with *ATP6V1B1* mutations. Only 1 patient with *ATP6V0A4* mutations had sensorineural hearing loss during childhood. Nine children developed medullary cysts, but without apparent clinical consequences. Cyst development in this cohort was not correlated with age at therapy onset, molecular diagnosis, growth parameters or renal function.

Conclusion: In general, the prognosis of dRTA is good in children treated with alkali.

Key words

Distal renal tubular acidosis, metabolic acidosis, hypokalemia, nephrocalcinosis, medullary cysts, gene mutation

Background

Distal renal tubular acidosis (dRTA), also often referred to as type 1 RTA, is caused by a decreased capacity of the α-intercalated cells in the collecting duct to excrete protons. Biochemically, it is characterized by a hyperchloremic metabolic acidosis with normal plasma anion gap, hypokalemia and hypercalciuria with hypocitraturia causing nephrocalcinosis. Despite a profound metabolic acidosis, patients are unable to acidify their urine and will have a urinary pH >5.5 [1,2].

Patients often present with vomiting, diarrhea or constipation, failure to thrive and/or rickets. Severe hypokalemia can cause muscle weakness or paralysis [2]. Chronic acidosis leads to bone demineralization which can result in rickets and/or stunted growth in children with dRTA. The excess of calcium that is released into the blood stream, combined with the decreased expression of renal calcium transporters in metabolic acidosis, causes hypercalciuria [3,4]. This hypercalciuria in turn can result in nephrocalcinosis, and if left untreated to renal calculi formation. Polyuria, due to impaired urinary concentrating ability (secondary nephrogenic diabetes insipidus) is another typical feature and may be related to electrolyte abnormalities in blood and/or urine and the nephrocalcinosis [5].

To date, several genes are known to cause dRTA. Autosomal recessive inheritance is noted in mutations in *ATP6V1B1*, *ATP6V0A4*, and sometimes *SLC4A1*. Mutations in the latter gene, however, generally cause the autosomal dominant form of dRTA [1].

In this study we present data on children with dRTA followed in our outpatient clinic over the last 20 years, including the available data of DNA analysis.

Patients and methods

This is a single centre, retrospective study on the molecular diagnosis and clinical follow-up of children with dRTA, followed in the specialized tubulopathy outpatient clinic at Great Ormond Street Hospital (London, UK) in the last 20 years. Distal renal tubular acidosis was defined as hyperchloremic metabolic acidosis with a normal plasma anion gap, with inappropriately high urinary pH on repeated sampling. All parents of children with dRTA were

offered genetic analysis to confirm their diagnosis. Clinical and biochemical data were retrieved from the patients' medical records and included details until transition into adult services. Estimated glomerular filtration rate (eGFR) was measured using the bedside Schwartz formula [6].

Results

In total 24 patients were included, with a median follow-up of 10 years (range 1-18 years). Clinical details are presented in table 1. The molecular diagnosis was confirmed in 19/24 patients. Six patients had homozygous or compound heterozygous mutations in *ATP6V1B1*, 10 had mutations in *ATP6V0A4* (including one pair of siblings) and 3 patients were found to have mutations in *SLC4A1*. We found 5 mutations not previously reported. These include 2 mutations in *ATP6V1B1* (c.905G>C; p.(Arg302Pro) in patient 2 and c.490_499del; p.(Ile164Alafs*8) in patient 6) and 3 mutations in *ATP6V0A4* (c.1312_1315del; p.(Asp438Metfs*13) in patient 9, c.970del; p.(Glu324Argfs*22) in patient 11 and c.1238G>A; p.(Gly413Asp) in patient 15). In patient 20 only one mutation was found in *ATP6V0A4*. Although this mutation was predicted to be pathogenic, he was not considered as having a confirmed molecular diagnosis since mutations in this gene have an autosomal recessive pattern of inheritance. Patients 5 and 15 were seen in our clinic for a second opinion, treatment strategies may therefore not necessarily represent the general practice in our hospital.

<u>Disease presentation</u>

Age at diagnosis

In general, patients with autosomal recessive mutations (patients 1-16 and patient 19) had a tendency to present earlier in life compared to patients with an autosomal dominant form of dRTA (patients 17 and 18). The children with autosomal recessive inherited mutations presented at a median age of 3 months (range 2 weeks – 2 years and 2 months), only 3 out of 17 children presented after the age of 1 year. In contrast, both children with autosomal

dominant mutations presented at after the age of 1 year, at 1 year and 5 months and 4 years, respectively (figure 1). This difference was statistically significant ($p<0.01$, student T-test).

Interestingly, patient 17 was assessed initially at the age of 1 year because of the family history of dRTA in both mother and maternal grandmother. Molecular diagnosis was not available in the family at the time and the initial clinical assessment was unremarkable with normal plasma electrolytes (K+ = 4.3 mmol, HCO3 = 20 mmol/l), normal venous pH (7.34), normocalciuria (calcium/creatinine ratio of 0.36 mmol/mmol) and normal renal ultrasound. A diagnosis was not established at the time and only further observation recommended. At 17-months of age, she had an episode of diarrhea and subsequent blood tests revealed acidosis (venous pH 7.29, plasma HCO3 16 mmol/l), with inappropriately elevated urine pH (7.5), revealing her to be affected, later confirmed by molecular diagnosis.

Presenting symptoms

Many children with dRTA presented with failure to thrive and growth retardation. This was most profound in the children with *ATP6V1B1* mutations and in those in whom no genetic mutation could be found (figure 2). Severe growth retardation according to the first documented measurement after presentation, with a height on or below -2 standard deviations scores (SDS), was found in a total of 12 children. It was documented in 4/6 children with mutations in *ATP6V1B1*, in 3/10 children with mutations in *ATP6V0A4*, in 1/3 with *SLC4A1* and in 4/5 children without a confirmed genetic diagnosis.

All children were found to have metabolic acidosis at initial presentation, but given the retrospective nature of this study the exact blood pH at diagnosis could only be recovered in 9 patients; all were <7.25. Serum bicarbonate levels at diagnosis, however, could be recovered in the majority of patients and were well below 18 mmol/L. Both blood pH and serum bicarbonate levels were the lowest in children with mutations in *ATP6V0A4*, children with mutations in *SLC4A1* generally presented with less severe acidosis (figure 3).

Two thirds of our patients presented with features of partial renal Fanconi syndrome. Fourteen children had tubular proteinuria, 8 children had a decreased tubular resorption of

phosphate (TRP) and 5 patients showed mild generalized aminoaciduria. None of the children had glycosuria. Surprisingly, at first presentation, only 7/24 children had an elevated urinary calcium/creatinine ratio. However, these measurements were performed on spot urine samples, and patients were polyuric, which in our laboratory can result in inaccuracies in the determination of creatinine in urine, as the assay is optimized for higher creatinine concentrations. A formal 24-h urine calcium determination would have been more informative, but is difficult to obtain in non-toilet trained children without catherization.

All patients except for patient 17 had nephrocalcinosis at first presentation, the first ultrasound in patient 5 was performed abroad and the report could not be retrieved. Patient 17 had a family history of autosomal dominant dRTA (in her mother and grandmother) and was screened before the onset of symptoms. In the other children, at the moment of diagnosis nephrocalcinosis was reported by the radiologist to be mild (increased echogenicity in the borders of the pyramids) in 10 patients, moderate (diffuse increased echogenicity of the entire pyramids) or moderate to marked in 4 children and marked (homogenous increased echogenicity of the entire pyramids, often in the presence of acoustic shadowing) in another 8 patients. Only 3 children developed renal calculi (patient 10, 19 and 23), none of them required medical intervention. There was no correlation with the degree of nephrocalcinosis at first presentation (marked in patient 10 and 23 and mild in patient 19).

Treatment

Treatment with alkali (bicarbonate and/or citrate) was started at a median age of 4 months (range 2 weeks – 5 years). Initial alkali prescription was as high as 8.6 mmol bicarbonate equivalent/kg/day in some children at the onset of therapy in order to achieve normal plasma bicarbonate values. In general, most children had a higher alkali requirement in the first years of life, which decreased after the first decade. Moreover, children with mutations that encoded part of the vacuolar H^+-ATPase (*ATP6V1B1* and *ATP6V0A4*) were prescribed

higher alkali doses compared to children with mutations that encode the anion exchanger 1 (*SLC4A1*), (figure 4).

Thiazide diuretics were administered to 3 patients. In patient 8 thiazides were started at the age of 2 months and stopped after two years, in patient 9 thiazides were started at the age of 1 month and stopped one month later. In both of them, thiazides were started in the referring centres. Patient 23 was started at the age of 11 years after two episodes of flank pain that resolved after passing concrements, Thiazide treatment was continued into adulthood.

Patient 15 was started on indomethacin by the referring team at the age of 6 months because of severe polyuria with the possible diagnosis of renal Fanconi syndrome. After the diagnosis of dRTA the indomethacin had been stopped at the age of 2 years, but was restarted because of worsening of polyuria.

Clinical features at last follow-up

Growth parameters

After the start of alkali treatment, there was a clear improvement in growth parameters in most children. Sustained growth retardation, with a height on or below -2 SDS on the most recent measurement, was documented in only 3 children, while the overall height SDS had improved in all groups. A similar improvement in weight SDS was found (figure 2). There was no clear correlation with growth retardation at the most recent follow-up and the age of therapy onset (2 months, 9 months and 5.3 years, respectively) or gene mutation (*ATP6V0A4*, *SLC4A1* and unknown). One child with sustained growth retardation had a normal renal function with an eGFR of 152 mL/min/1.73 m^2, the other two had chronic kidney disease (CKD) stage 2 with an eGFR of 67 and 70 mL/min/1.73 m^2, respectively.

Chronic kidney disease

Renal insufficiency was found in 9/24 children, all of them had an eGFR of 60-90 mL/min/1.73 m^2, consistent CKD stage 2. There was no clear correlation with renal function

and genetic diagnosis: decreased renal function was found in 1/6 patients with mutations in *ATP6V1B1*, in 5/10 patients with *ATP6V0A4* mutations, in 1/3 patients with *SLC4A1* mutations and in 2/5 children who had no confirmed genetic diagnosis.

Radiological features

In 9/24 patients, medullary cysts were reported on the most recent renal ultrasound. The age at which the first cysts were noted ranged between 3-17 years. Two out of three patients who developed renal calculi had medullary cysts, but there was no correlation with the degree of nephrocalcinosis at diagnosis (which was mild in 5 patients with medullary cysts, moderate in 1 and marked in another 3 patients). Also, there was no clear effect on renal function, which was normal in 3 patients with renal cysts while the remaining 6 patients had CKD stage 2. Four out of ten patients with *ATP6V0A4* mutations developed cysts, all after the age of 10. Two out of three patients with mutations in *SLC4A1* developed cysts, one was 4 years old and the other at the age of 17. No molecular diagnosis could be made in the remaining 3 patients that developed medullary cysts, all of them were below the age of 10 when the cysts were first noted. There was no apparent correlation between the degree of nephrocalcinosis and the development of CKD.

Hearing

Hearing loss was documented in 11 children, including all children with *ATP6V1B1* mutations. The hearing problems were found to be sensorineural in origin in 6 patients, 5 of which had documented mutations in *ATP6V1B1*. In the other child no causative mutations were found. Three children were diagnosed with conductive hearing loss. This was attributed to recurrent ear infections in 2 of them and improved over time, in the other child no mutation was found. Another 2 children were found to have combined sensorineural and conductive hearing problems, one of them had a mutation in *ATP6V0A4* and the other one in *SLC4A1*.

Discussion

We present clinical data on the follow-up of children with dRTA in a single centre, including the available data on genetic analysis. Although the sample size is rather small, this study contains valuable data on the long-term follow-up of patients with a rare disease. The majority of patients were followed for a long period of time, 20/24 patients were followed for at least 5 years and 12/24 patients for at least 10 years. The addition of molecular diagnostics enables us to compare the clinical phenotype with the molecular genotype. Moreover, the analysis of clinical data informs the prognosis and management of patients with this rare disease.

Lastly, we found 5 mutations that to our knowledge are new to the literature. Publication of these will help establishing the diagnosis in other patients found to carry these mutations.

Treatment

The administration of alkali is the cornerstone of treatment of dRTA. Due to a higher metabolic rate and protein intake in growing children, they may need relatively higher treatment doses than adults when corrected for body weight to maintain a normal pH. This was previously reported in a historical study in which 5 children with dRTA were followed for a period of <10 years [7]. Where adults in general require 0.5-1 mEq/kg/d of bicarbonate, this can be up to 5-8 mEq/kg in children [1]. The fact that younger children in general need higher doses of alkali was confirmed in this study. Moreover, children with mutations interrupting the function of the vacuolar H^+-ATPase generally needed higher doses of alkali compared to those with mutations in *SLC4A1* (figure 4) and prescribed doses were typically highest at therapy onset. This is in line with the finding that children with mutations in *ATP6V0A4* in general had a lower blood pH and lower serum bicarbonate levels at diagnosis (figure 3). In cases with severe hypokalemia, potassium supplements may also be warranted. In these patients, the prescription of potassium citrate or potassium bicarbonate can prove to be an elegant way to administer both.

Thiazide diuretics are sometimes used to decrease urinary calcium excretion, but they can in turn increase the risk of hypokalemia and polyuria and are thus not routinely used in our centre. In our cohort of 24 patients, only 3 children developed small calculi and none of them needed an intervention. Moreover, urinary calcium excretion is increased in acidosis and decreases after the administration of alkali [7,8]. This makes it a good marker for adherence to and/or adequacy of treatment, but is no longer reliable after the administration of thiazide diuretics. Thus, in our opinion the use of alkali treatment, sometimes with additional potassium supplements, is sufficient to treat the majority of children with dRTA.

The experience with patient 17, in whom routine biochemistries of plasma and urine were normal at the age of 12 months, suggests that a clinical diagnosis of dRTA cannot be excluded at such a young age in at-risk children and should ideally be established molecularly, or, if this not possible, by clinical examination when the system is “stressed” (for instance after acid loading or, as in patient 17, during an episode of gastroenteritis with intestinal bicarbonate loss).

Associated symptoms

Interestingly, 16/24 children in this study had features of renal Fanconi syndrome at presentation. The latter indicates a proximal tubular dysfunction characterized by low-molecular weight proteinuria (also referred to as tubular proteinuria), generalized aminoaciduria, hyperphosphaturia, glucosuria and increased urinary losses of bicarbonate (causing metabolic acidosis). Several other solutes that are reabsorbed in the proximal tubule can be lost as well [9,10]. In our cohort, the 16 patients with partial renal Fanconi syndrome showed a combination of low-molecular weight proteinuria (measured as urinary retinol binding protein/creatinine ratio), increased urinary losses of phosphate (measured as TRP) and/or mild generalized aminoaciduria. Metabolic acidosis was obviously found in all patients, since this is a key feature of dRTA. However, none of the patients had a full-blown renal Fanconi syndrome, since none of them showed glucosuria. In this way, the proximal tubulopathy resembles that of Lowe syndrome or Dent disease [11]. After initiation of alkali

treatment, the features of renal Fanconi syndrome disappeared in all patients, establishing the correct diagnosis of dRTA in some children that had previously been diagnosed with renal Fanconi syndrome of unknown etiology. There have been several reports of children with dRTA presenting with partial renal Fanconi syndrome (low-molecular weight proteinuria, hyperphosphaturia and/or generalized aminoaciduria). As in our study, none of them had glucosuria and in all of them the proximal tubulopathy disappeared after treatment onset [7,12-14]. The exact mechanism by which dRTA causes proximal tubular damage is unknown, but given the similarity of the tubulopathy with Dent Disease/Lowe syndrome it is tempting to speculate that this may be due to impairment of similar pathways. Indeed, apart from those with unknown mutations the proximal tubulopathy was seen only in children with mutations in subunits of the vacuolar ATPase, which is expressed together with CLC-5 (the chloride transporter underlying Dent disease) in the lysosomes of the proximal tubule and α-intercalated cells [15]. However, why this lysosomal dysfunction would improve with correction of the acidosis is unclear, and the proximal tubulopathy may just reflect a general dysfunction from the acidosis. From a clinical point of view, the key conclusion is to not prematurely exclude dRTA as a potential diagnosis in patients with a proximal tubulopathy, especially in young children with associated nephrocalcinosis. After correction of the acidosis and hypokalemia the abnormal markers of proximal tubular function should be monitored, since they will normalize in children with underlying dRTA.

In our population of 24 children with dRTA, the majority of patients (16 in total) had proven mutations in *ATP6V1B1* (n=6) and *ATP6V0A4* (n=10), interrupting the function of the vacuolar H^+-ATPase. Both mutations can be associated with sensorineural hearing loss [1]. In fact, all 6 patients in our cohort who were found to have *ATP6V1B1* mutations also had hearing loss. However, this was conductive in origin in one of them and was attributed to recurrent ear infections. Only one child with mutations in *ATP6V0A4* was diagnosed with hearing loss: patient 13 was found to have a combined bilateral sensorineural and unilateral conductive hearing loss. Her sister, however, was not noted to have any hearing problems. Since both have the same mutation in *ATP6V0A4*, it remains unclear whether this mutation is

in fact causing the hearing loss in patient 13. It has been noted before that hearing loss in children with *ATP6V1B1* mutations generally presents before the age of 10 years, while hearing loss in patients with *ATP6V0A4* mutations is often only diagnosed from the second decade of life [16,17]. The outcome in our cohort is in line with these findings. It also warrants the need for regular hearing examination in patients without any signs of deafness at diagnosis, since sensorineural hearing loss can still occur later in life.

We present one child (patient 19) with the concomitant occurrence of Southeast Asian ovalocytosis and dRTA due to mutations in *SLC4A1*. The protein encoded by this gene is a polypeptide which forms a homodimer, to form the transporter AE1. The first deletion found in this patient is c.1199_1225del; p.(Ala400-Ala408del). This is a well-known mutation typically causing Southeast Asian ovalocytosis, in general occurring without a renal phenotype. Mutant non-functioning polypeptides form heterodimers with the wild type polypeptide. The minimal net effect on the transport function of the newly formed protein results in the absence of a renal phenotype in presence of a wild type allele. However, this patient had a second mutation in her other allele, being c.2102G>A; p.(Gly701Asp). This mutation gives rise to a mutant polypeptide that is trapped within the cell (probably in the Golgi apparatus), thus causing autosomal recessive dRTA. The presence of both mutations in 1 patient (one resulting in a non-functioning polypeptide and the other one resulting in a polypeptide being trapped intracellularly) causes autosomal recessive dRTA, while the c.1199_1225del mutation alone has been reported not to cause a renal phenotype [18,19]. Although very rare, this combination of c.1199_1225del with c.2102G>A has been reported before to cause a combination of Southeast Asian ovalocytosis and dRTA [20].

In this study, we found medullary cysts on the most recent renal ultrasound in 37.5% of our study population (9/24 patients). The development of medullary cysts in a subset of patients with dRTA has been well described [21]. The exact etiology of these cysts remains unclear, but again it was hypothesized that this may be caused by hypokalemic nephropathy. Besides the ischemic changes, hypokalemic nephropathy is associated with tubular hypertrophy and dilatation, which may cause renal cyst formation [22]. However, not all

conditions with chronic hypokalemia result in cyst formation. For instance, Bartter syndrome is often associated with severe chronic hypokalemia but medullary cysts are only rarely reported [23]. Moreover, in our population all patients had developed cysts while they were receiving treatment. There was no correlation with the development of medullary cysts and the degree of nephrocalcinosis at diagnosis, nor with renal function at last follow-up. The development of medullary cysts is thus noted in a subset of patients with dRTA, but the clinical consequences remain limited in our cohort.

Conclusion

In general, the prognosis of dRTA is good when the diagnosis can be made early and if there is a good compliance to alkali treatment [2]. In this study, we did not find a correlation between the age at therapy onset or gene mutation and stunted growth or renal function. Nine patients were found to have CKD stage 2. Deterioration of renal function can be seen in patients with dRTA, mainly in those with hypercalciuria and recurrent renal stones, but progression into end-stage renal disease is only rarely reported [24]. Growth retardation is often noted at diagnosis in children with chronic dRTA and is attributed to chronic metabolic acidosis. However, after the initiation of alkaline treatment catch-up growth can be noted [2,7] and in this study most children (87.5%) had a height within normal limits for age at last follow-up, despite profound failure to thrive at diagnosis in 50% of them (figure 2). Thus, we can confirm the general good prognosis of dRTA in children under alkali treatment.

In conclusion, we present clinical data on long-term follow-up of 24 children with dRTA in a single center, including available data on genetic screening and on 5 novel disease causing mutation. All but 1 patient presented with the combination of metabolic acidosis and nephrocalcinosis, 16 children had features of proximal tubular dysfunction which disappeared after initiation of treatment. The diagnosis of renal Fanconi syndrome should be made with care in children with severe metabolic acidosis and nephrocalcinosis at a young age; markers of proximal tubulopathy should be followed after initiation of treatment. Failure to thrive is often noted at diagnosis, alkali treatment results in improved growth

parameters in most children. Although their clinical relevance remains uncertain, medullary cysts were found in 6 children and are often reported in patients with dRTA. Also, hearing loss is often associated with dRTA, mainly when mutations are documented in *ATP6V1B1* or *ATP6V0A4*. In the latter gene, sensorineural deafness often develops in the second decade of life, warranting long-term follow-up of hearing in children with dRTA with normal initial hearing screening who have mutations in *ATP6V1B1*, *ATP6V0A4* or in whom no mutation was found. The basic treatment of dRTA consists in the prescription of alkali, if necessary combined with potassium supplementation. Although there was a tendency in children with *ATP6V1B1* or *ATP6V0A4* mutations to present earlier in life and to need higher doses of alkali, our study population was rather small and we found no statistical significant correlation between gene mutation, age at therapy onset, growth parameters or renal function. Long-term studies including genetic analysis in a bigger population, including data before and after transition into adult care can further elaborate on the effects of early treatment onset and gene defect on final height, deterioration of renal function over time and the development of nephrocalcinosis with calculi and medullary cysts.

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Figure 1. Age at onset of alkali treatment.

Boxplot showing the age in months when alkali treatment was first initiated, in the different genotypes on the left and of all patients on the right.

Figure 2. Height and weight at diagnosis and at most recent follow-up.

Boxplot showing the height SDS (upper panels) and weight SDS (lower panels) at diagnosis (left panels) and at most recent follow-up (right panels). In each panel the different genotypes are depicted on the left and the mean of all patients is depicted on the right.

Figure 3. Blood pH and serum bicarbonate levels at diagnosis.

Boxplot showing blood pH (left panel) and serum bicarbonate (right panel) at diagnosis. In each panel the different genotypes are depicted on the left and the mean of all patients is depicted on the right.

Figure 4. Alkali treatment (equivalent to mmols of bicarbonate/kg/day) at different ages.

Boxplot showing the alkali requirement at the age of 1, 5, 10 and 15 years. The first four panels represent the alkali requirement per genotype, the last panel represents the alkali requirement in all patients.