Nephrology Dialysis Transplantation

Case Report

CLCN5 mutation (R347X) associated with hypokalaemic metabolic alkalosis in a Turkish child: an unusual presentation of Dent's disease

Nesrin Besbas[1], Fatih Ozaltin[1], Nikola Jeck[2], Hannsjörg Seyberth[2] and Michael Ludwig[3]

[1]Department of Pediatrics, Unit of Nephrology, Hacettepe University Faculty of Medicine, Ankara, Turkey,
[2]Department of Pediatrics, Philipps University of Marburg and [3]Department of Clinical Biochemistry, University of Bonn, Bonn, Germany

Keywords: chloride channel 5 (ClC-5); *CLCN5* gene; Dent's disease; metabolic alkalosis

Introduction

Dent's disease, an X-linked recessive tubular disorder, is characterized by low molecular weight proteinuria (LMWP) and nephrolithiasis associated with nephrocalcinosis and hypercalciuria. It is due to mutations that inactivate the renal voltage-gated chloride channel ClC-5 [1,2], which is encoded by a gene (*CLCN5*) located on chromosome Xp11.22. It is possible, however, that causative mutations were not identified in some patients with Dent's disease [3–6]. Renal acidification abnormalities have not been a consistent feature of the phenotype, probably being secondary to long-standing hypercalciuria and nephrocalcinosis. Hypokalaemic metabolic alkalosis, however, has not been reported previously in Dent's disease.

Inherited disorders that manifest hypokalaemic metabolic alkalosis, such as the Bartter–Gitelman syndrome or the hyperprostaglandin E syndrome (also referred to as antenatal Bartter's syndrome), are caused by the malfunction of renal tubular electrolyte transporters or ion channels. The hyperprostaglandin E syndrome is linked to the dysfunction of the sodium–potassium–chloride co-transporter (NKCC2) [7] or the renal outer medullary potassium channel (ROMK) [8]. The cardinal features of the syndrome are its antenatal onset—with polyhydramnios due to fetal polyuria, isothenuria and medullary nephrocalcinosis. When associated with sensorineural deafness or autosomal dominant hypocalcaemia, the hyperprostaglandin E syndrome is due to mutations in barttin, a β sub-unit of voltage-gated chloride channels [9], and the calcium-sensing receptor CaSR [10]. Bartter–Gitelman syndrome is linked to mutations in the basolateral chloride channel (ClC-Kb) [11] or in the sodium–chloride co-transporter (NCCT) [12]. The course of this disease is usually milder, mimicking chronic use of thiazides. ROMK, NKCC2 and NCCT mutations usually have uniform clinical presentations, whereas mutations in *CLCNKB*, encoding ClC-Kb, occasionally lead to phenotypic overlaps with the ROMK/ NKCC2 cohort.

This study describes the first case of Dent's disease due to a loss-of-function mutation in the *CLCN5* gene, R347X, associated with a Bartter-like syndrome that is characterized by hypokalaemic metabolic alkalosis and secondary hyper-reninaemic hyperaldosteronism.

Case

The 4-year-old son of unrelated parents was admitted to our centre due to urolithiasis. There was no family history of renal disease, including renal stone disease. The boy's physical examination was normal, including blood pressure (100/60 mmHg), and there was no evidence of growth retardation. Biochemical analyses showed a normal glomerular filtration rate (97 ml/min/ 1.73 m^2) and normal levels of calcium, magnesium, uric acid and sodium; however, serum potassium (2.3 mEq/l) and phosphorus (2.6 mg/dl) were low. Serum magnesium was normal (2.2 mg/dl). In addition, we discovered that the patient was in metabolic alkalosis (pH 7.52, HCO$_3$ 33 mmol/l), while his urine was alkaline (pH 8), with a low specific gravity (1004). Subsequent investigations showed proteinuria (49 mg/ m^2/h) and hypercalciuria (7.6 mg/kg/day), which were confirmed on multiple random urine specimens. Urinary protein electrophoresis showed increased α-1, α-2 and β peaks compatible with tubular proteinuria. The urinary amino acid profile showed no abnormality. The tubular reabsorption of phosphate was 80%. Urinary electrolytes were as follows: Cl 56 mEq/l,

Correspondence and offprint requests to: Nesrin Besbas, MD, Hacettepe University Faculty of Medicine, Department of Pediatric Nephrology, 06100 Sihhiye, Ankara, Turkey. Email: nbesbas@ hacettepe.edu.tr

Na 50 mEq/l, K 40 mEq/l. Oxalate and citrate levels in the 24 h urine were normal. Serum parathyroid hormone (22.3 ng/ml, N 12–72) as well as 25-hydroxy vitamin D levels (22.73 mg/ml, N 10–68) were normal, in contrast to very high renin (588 pg/ml) and aldosterone (946 pg/ml) levels. Medullary nephrocalcinosis was documented by ultrasound examination. The patient's hearing was normal (as it was on every subsequent annual testing). Bone mineral densitometry showed mild demineralization (z score −0.83). The β_2-microglobulins of the urine of the patient's mother and brother were within normal limits; and neither of them was hypercalciuric (urine calcium <4 mg/kg/day).

Gene analysis in the patient included the genes coding for: (i) the chloride channel 5 (*CLCN5*); (ii) the thiazide-sensitive Na^+/Cl^- co-transporter (*SLC12A3*); (iii) the renal chloride channel (*CLCNKB*); (iv) the Na–K–2Cl co-transporter (*SLC12A1*); and (v) the renal outer medullary K^+ channel (*KCNJ1*). In order to detect the mutation responsible for our patient's disease, we initially amplified all exons (with their corresponding splice sites) of the genes encoding ClC-5, NCCT and ClC-Kb. Sequence analysis of the resultant polymerase chain reaction (PCR) products revealed only one deviation from the wild type in the PCR product corresponding to the *CLCN5* exon 8. The sequence variation detected in this sample turned out to be a hemizygous C1330T transition which codon 347 (CGA: arginine) changed to a premature stop codon. [Nucleotide numbering is according to the cDNA (accession no. NM_000084) with the A of the start-ATG at nucleotide 292.] This C1330T substitution was not present in samples from 50 Caucasian controls (25 males and 25 females = 75 alleles), and family analysis revealed that the patient had inherited this mutant allele from his asymptomatic carrier mother.

To elucidate further the aetiology of the patient's phenotype, sequence analysis was also performed for his genes encoding ROMK and NKCC2; however, no abnormalities could be detected. Aside from known *CLCNKB* polymorphisms (see single nucleotide polymorphism data listed in the Ensemble Human Genome Browser), we also detected two novel intronic variants in the patient: in intron 5, a homozygous C to T transition was observed at position −5 of the acceptor splice site of exon 6 (IVS5 −5), and the intron 10 position +13 (IVS10 +13) was found to be heterozygous (G/C). Since aberrant *CLCNKB* mRNA splicing would provide an explanation for the phenotype observed in our patient, these variants were tested not only for their occurrence in a panel of normal control subjects, but also for their potential to affect normal splicing. The initial restriction analysis of the respective PCR products showed both variants to be true polymorphisms: we observed allelic frequencies of 0.78 (T) and 0.22 (C) in IVS5 −5, and of 0.63 (C) and 0.37 (G) for the IVS10 +13 variant. We then analysed *CLCNKB* mRNA transcripts from subjects homozygous for each of these polymorphisms. However, in all cases, the amplification of products
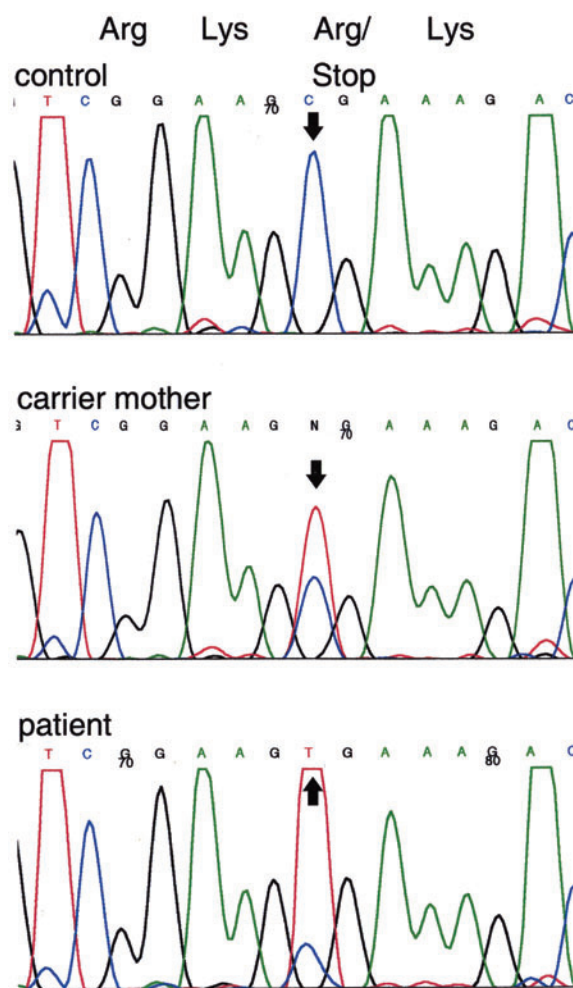
Fig. 1. *CLCN5* gene analysis. The sequence shown, of part of exon 8 and the hemizygous C1330 to T substitution (Arg347→stop) observed in the patient (bottom), is indicated by an arrow. This mutation could also be observed in the heterozygous state (middle, indicated by N, arrow) in the mother, and thus establishes her carrier status.

attributable to exons 5–7 and 9–12/13 revealed only the normal message and provided no evidence of exon skipping or cryptic splice site usage (data not shown).

Treating our patient with indomethacin and thiazide diuretic and potassium and phosphorus supplementation resulted in the normalization of plasma ion abnormalities and polyuria. His hypercalciura was resolved (1.8 mg/kg/day) and renal stones did not recur through 4 years of follow-up.

Discussion

For the first time in the medical literature, we report here a child with Dent's disease due to a loss-of-function mutation in the *CLCN5* gene, R347X, associated with a Bartter-like syndrome that is characterized by hypokalaemic metabolic alkalosis and hyper-reninaemic hyperaldosteronism.

Inherited disorders that manifest hypokalaemic metabolic alkalosis such as the Bartter–Gitelman and hyperprostaglandin E syndromes are caused by the malfunction of renal tubular electrolyte transporters or ion channels. All variants share several clinical characteristics, including renal salt wasting, hypokalaemic metabolic alkalosis and hyper-reninaemic hyperaldosteronism. Our patient presented with hypokalaemic metabolic alkalosis associated with hyper-reninaemic hyperaldosteronism, hypercalciuria and nephrocalcinosis, but without antenatal history, thereby suggesting Bartter's syndrome. However, we also identified LMWP, which is not a feature of Bartter's syndrome, but which is the most consistent laboratory finding in Dent's disease. This raises the question of whether or not both Bartter's syndrome and Dent's disease might be present in the same patient.

It is known that mutations in NCCT, ROMK and NKCC2 usually have uniform clinical presentation, whereas mutations in *CLCNKB*, encoding ClC-Kb, occasionally lead to phenotypic overlaps with the ROMK/NKCC2 cohort. For this reason, we searched not only for *CLCN5* mutations but also for defects in the genes encoding NCCT, ClC-Kb, ROMK and NKCC2. However, apart from the identification of the *CLCN5* exon 8 mutation (R347X), we did not detect any mutations in these genes from our patient. We only observed two novel intronic *CLCNKB* nucleotide substitutions—which were found to represent neutral polymorphisms that do not interfere with normal proper splice site recognition. Since the IVS10 $+13G > C$ variant does not affect the donor motif of exon 10, and no consensus value (CV) could be predicted, these findings are consistent with the CV obtained for the acceptor splice site variation in intron 5 (IVS5 $-5C > T$) in our patient. Here, nearly identical values could be calculated (type C, ctcggatcccccag A, CV = 0.813; type T, ctcggatcctccag A, CV = 0.823; where the substitution is underlined, and indicates the splice site, with the exonic A in upper case); and the T value observed in our patient was even better.

Other rare variants of inherited hypokalaemic salt-losing tubulopathies could be excluded in our patient clinically. The onset of the disorder was after infancy, which is unusual for the hyperprostaglandin E syndrome. In addition, he did not have a sensorineural deafness, which is invariably linked to barttin mutations, and a CaSR defect would be unlikely due to the absence of hypocalcaemia. However, compared with the X-linked *CLCN5* gene, the mutation might have been missed due to the failure to amplify the respective PCR product from one of the two alleles in the tested autosomal genes (*SLC12A1*, *SLC12A3*, *KCNJ1* and *CLCNKB*). Also, the failure to detect mutation could be due to the fact that the regulating elements of these genes (elements located in intronic regions or proximal or distal to these genes) either were not investigated or the amplification technique will not identify deletions or inversions, or both. On the other hand, the absence of mutations in the patient we studied does not exclude the possibility that any of these genes could be

Table 1. Comparison of the clinical features of our patient and of two Japanese patients

	Akuta *et al.* [3]	Morimoto *et al.* [4]	Present case
Age (years)	7	12	4
Gender	M	M	M
Serum			
Cr (mg/dl)	0.3	0.9	0.5
K (mEq/l)	N/A	N/A	2.3
Cl (mEq/l)	109	N/A	94
Ca (mg/dl)	9.8	9.2	9.8
P (mg/dl)	5.8	5.1	2.6
pH	N/A	N/A	7.52
HCO_3 (mmol/l)	N/A	N/A	33
Urine			
Protein	2+	2+	2+
β_2-microglobulin (µg/l)	130 000	64 800	44 330
Occult blood	±	1+	±
Ca/Creat	NT	0.3	0.34
Urinary Ca excretion (mg/kg/day)	N/A	N/A	7.6
Nephrocalcinosis	No	No	Yes
Renal stones	No	No	Yes

N/A = not available; NT = not tested.

implicated in other patients with phenotypes resembling the Bartter–Gitelman or hyperprostaglandin E syndromes.

The R347X mutation has been reported previously in two Japanese patients, where it was associated with a milder phenotype than we observed in our patient [3,4]. These Japanese children had LMWP and slightly increased calcium excretion, but no nephrocalcinosis. Younger age, hypokalaemic hypochloraemic metabolic alkalosis, hypophosphataemia, nephrocalcinosis and nephrolithiasis without osteopenia and rickets are the main features which are different in our case compared with these two patients (Table 1). A third report described an American patient with the R347X mutation [5]; and here, although no clinical details were given, a fuller phenotype of Dent's disease was manifest. This reflects the general observation that a single *CLCN5* mutation can be associated with a range of phenotypes.

ClC-5 expression in human nephron occurs intracellularly, in the subapical endosomes of kidney epithelial cells lining the proximal tubules, the medullary thick ascending limbs of Henle's loop and the intercalated cells of the collecting ducts. Thus, ClC-5 dysfunction in the proximal tubule may result in low molecular weight proteinuria together with features of Fanconi syndrome, and ClC-5 dysfunction in the thick ascending limb of Henle, which is the major site of calcium reabsorption, may result in hypercalciuria [2]. Hypercalciuria is the major risk factor promoting stone formation in Dent's disease. It has been shown that the inactivation of the ClC-5 chloride channel does not impair calcium transport in the distal convoluted tubule, due to an intact hypocalciuric response to thiazide diuretics [13]. We therefore used a thiazide

diuretic in our patient; and did not observe hypercalciuria or recurrence of kidney stones through 4 years of follow-up.

Chloride efflux across the basolateral membrane of the thick ascending limb can occur mainly via the ClC-Kb channel, but also via other chloride channels, such as the highly homologous ClC-Ka channel, CFTR, and ClC-5. Also, members of the potassium chloride co-transporter family are localized in this nephron segment. Hence, a defect in the activity of ClC-Kb is expected to reduce NaCl reabsorption in these tubule segments, but does not abolish salt reabsorption completely. Defects in any of these proteins would impair net NaCl reabsorption in the thick ascending limb, and thereby increase NaCl delivery to more distal nephron segments—whose consequence is salt-wasting volume contraction and stimulation of the renin–angiotensin–aldosterone axis, which leads to hypokalaemic metabolic alkalosis. The metabolic alkalosis of our patient was most probably due to this mechanism.

It has long been known that Bartter's syndrome may be secondary to other familial disorders that affect the kidneys, such as nephropathic cystinosis, Kearns–Sayre syndrome, Menke's kinky hair syndrome and familial renal dysplasia. Drugs—the chronic use of diuretics and aminoglycosides and chemotherapy—can mimic Bartter–Gitelman syndrome. Since none of them was in use in our patient at the time of diagnosis, we excluded these secondary causes of that syndrome.

In conclusion, in metabolic alkalosis accompanied by Dent's disease, one or more additional mutations in one or more other genes may provoke a disorder that 'phenocopies' other tubular disorders. The co-existence of hypokalaemic metabolic alkalosis and LMWP associated with renal stone disease should prompt clinicians to search for Dent's disease.

Conflict of interest statement. None declared.

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