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ORIGINAL ARTICLE

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Type IV Bartter syndrome: report of two new cases

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Abstract Bartter syndrome with sensorineural deafness (type IV Bartter syndrome) is a subtype of this tubular disease, and is due to mutations in the *BSND* gene. Out of a population of 92 patients with Bartter syndrome, five suffered from mild to severe hypoacusia and were selected for mutational screening. A homozygous mutation in the *BSND* gene was found in two female patients. The first patient was found to have a substitution in intron 1 donor splice site at position +5 (c.420+5G>C), whereas the second patient has a homozygous 3G>A substitution leading to the loss of the start codon for the translation of the *BSND* mRNA. The clinical courses of these two patients were remarkable for severe polyhydramnios, massive renal salt and water wasting, severe neonatal hypotonia, poor growth and unresponsiveness to prostaglandin inhibitors. The diuretic responses to furosemide and to hydrochlorothiazide were tested under KCl supplementation in one patient. A lack of response to both drugs suggested that inhibition of NaCl reabsorption in type IV Bartter syndrome is not restricted to the thick ascending limb of Henle. In one patient, a combined therapy with indomethacin and captopril was needed to discontinue intravenous fluids and improve weight gain.

Keywords Bartter syndrome · Sensorineural deafness · Captopril · Indomethacin · Nimesulide · Furosemide · Hydrochlorothiazide

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Introduction

Bartter syndrome (BS) identifies a group of closely related hereditary salt-losing renal tubular disorders characterized by hypokalemic metabolic alkalosis. To date, five different genes involved in NaCl reabsorption in the thick ascending limb of Henle have been identified [1].

Infantile BS with sensorineural deafness, also referred as type IV BS (OMIM 602522), is in most cases due to autosomal recessive mutations in the *BSND* gene, located on chromosome 1p31 [2–4]. The *BSND* gene encodes for the Barttin protein, which acts as an essential subunit of the ClC-Ka and ClC-Kb chloride channels. In the kidney, Barttin is expressed in tubular segments spanning from the thick ascending limb to cortical collecting ducts, whereas in the inner ear, it is expressed in potassium-secreting epithelial cells [5].

Most mutations of the *BSND* gene cause a severe form of neonatal BS which is characterized, in addition to deafness, by markedly severe tubular salt wasting, frequent evolution to chronic renal failure, absence of overt medullary nephrocalcinosis and resistance to indomethacin therapy [6]. Altogether, *BSND* mutations are responsible for a minority of the cases of BS. To date, only a few mutations have been reported [2].

Methods and patients

Patient selection

All BS patients with hearing impairment were selected in order to screen for mutations in the *BSND* gene. A hearing test was not performed for all patients; only those that had a neonatal form of BS or had clinical evidence of hearing impairment were tested.

Genetic analysis

DNA was extracted from blood samples using the QIAamp DNA blood Minikit (QIAGEN S.p.A., Milano, Italy). Exons 1–4 of the *BSND* gene were amplified by polymerase chain reaction (PCR) using previously published primer sequences and amplification conditions [2]. Direct sequencing was performed with the Big Dye Terminator kit (Beckman Coulter, Brea, CA, USA) and resolved by capillary electrophoresis with a CEQ2000XL DNA Analysis System (Beckman Coulter). Restriction fragment length polymorphism was performed at 37 °C for 3 h with the *Alu*I restriction enzyme (Fermentas, Burlington, Ontario, Canada) on PCR fragments amplifying exon 1 flanked by 35 additional bases. The digested fragments were resolved on a 2% agarose gel.

Hydrochlorothiazide and furosemide testing

Furosemide (0.5 mg/kg) or hydrochlorothiazide (0.5 mg/kg) [7] were given per os on different days, 30 minutes after KCl supplementation. To avoid any worsening of hypokalemia, intravenous KCl infusion was given through an existing intravenous line. Two baseline urine samples were collected at one-hour intervals before the test, and one and two hours after diuretic administration. Serum K was monitored after two and four hours. Written informed consent was obtained.

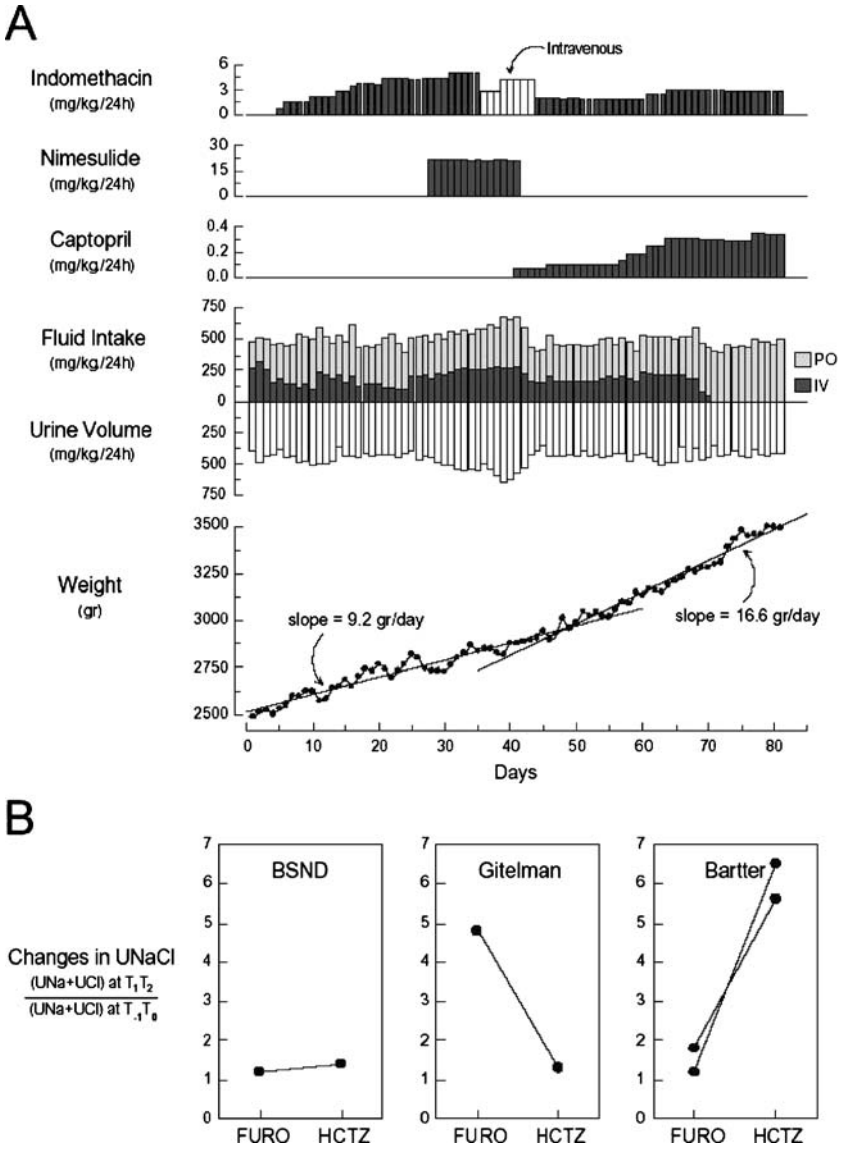

Fig. 1 **a** Fluid balance, therapy and weight gain of patient 1. **b** Renal response to furosemide and to hydrochlorothiazide in patient 1 (*BSND*), in one patient with Gitelman syndrome and one patient with Bartter syndrome. See text for details

Urinary Na, Cl and creatinine were measured in all collected samples. The [NaCl]/creatinine ratio (mmol/mmol of creatinine) was averaged in the two baseline samples and in the two test samples. Results were expressed as relative changes in urine [Na+Cl] excretion from baseline.

Results

BSND gene mutations

Out of a cohort of 92 patients with BS followed in Milano and Rome, five patients had evidence of hearing impairment and were screened for *BSND* mutations. Mutations were found in two patients. One of the three patients without detectable mutations was subsequently diagnosed with a mitochondria cytopathy. The second patient developed a severe psychotic disorder associated with her BS over time, and the third patient presented with only mild hearing impairment, which may have been secondary to drug toxicity during the neonatal period. Genetic testing for other BS genes is underway.

One patient (patient 1) was found to have a novel homozygous mutation in the intron 1 donor splice site at position +5 (c.420+5G>C). According to the "*Automated Splice Site Analyses program*" (http://splice.cmh.edu/), this mutation is predicted to cause an 8.3-fold decrease in the binding site strength, resulting in a complete loss of the splice site. Unfortunately, amplification of *BSND* mRNA from shedded renal tubular cells recovered from urine samples was unsuccessful, whether attempted from the patient's urine or from the urine of control subjects.

As this mutation introduces a restriction site for the *Alu*I enzyme, screening for a potential polymorphic change was achieved by restriction fragment length polymorphism. No mutation was found in 200 chromosomes from normal Italian individuals. Both parents were found to be heterozygous carriers.

The second patient (patient 2) was found to have a homozygous 3G>A substitution leading to the loss of the start codon (ATG mutated into ATA) of the *BSND* mRNA. Both parents were found to be heterozygous carriers.

In both cases the parents were unrelated, but they came from families from the same small province in northern or southern Italy.

Clinical evolution

A. Patient 1 was born prematurely at 33 weeks of gestation with a birth weight of 2.450 kg after a pregnancy complicated by severe polyhydramnios. Soon after birth, the child developed severe polyuria associated with marked hypokalemic alkalosis. She was transferred to our unit at one month of age. Her physical examination was remarkable for prominent axial hypotonia with spontaneous hyperextension of the neck. Initial serum blood tests showed the following results: creatinine 45.1 μmol/L, K 2.8 mmol/L, Na 132 mmol/L, Mg 0.62 mmol/L, HCO_3 38 mmol/L, BE +15 mmol/L, aldosterone >4.15 nmol/L, renin >7.58 pmol/L. Urinary calcium/creatinine ratio was within the normal range (1.7–2.3 mM/mM). The renal ultrasound showed diffusely hyperechogenic kidneys without signs of nephrocalcinosis. Hearing tests showed an absence of evoked response to stimulation up to 120 dB.

Figure 1a summarises the fluid balance, weight gain and response to therapy of patient 1 during the first few weeks after admission. Primary treatment included supplementation with fluids and electrolytes to match urinary losses, both intravenously and per os through a nasogastric feeding tube. Mean KCl and NaCl requirements were 27 and 34 mmol/kg/day respectively, while fluid intake averaged 526 ml/kg/day during the first two weeks.

Indomethacin treatment was immediately initiated using doses of up to 5 mg/kg/day, without obtaining significant clinical and biochemical improvement. No improvement was observed after additional therapy with the cyclooxygenase-2 inhibitor nimesulide [8] at a dose of 20 mg/kg/day either. Following the hypothesis that indomethacin was not efficiently reabsorbed by the gastrointestinal tract, it was also given intravenously for six days without any noticeable benefit.

Based on the resistance to prostaglandin inhibitors, the indomethacin dosage was lowered to 3 mg/kg/day and captopril was initiated at a dosage of 0.35 mg/kg/day. This treatment regimen was tolerated well and considerably improved the clinical course. The average weight gain increased from 9 to 16 g/day. Fluid requirements decreased from 530±66 to 473±44 ml/kg/day and urine output decreased from 479±63 to 430±40 ml/kg/day. Most noticeably, intravenous fluids could be stopped, which had been attempted unsuccessfully twice under the previous treatment regimens. The mean serum creatinine increased from 47.7±5.3 to 55.7±9.7 μmol/L ($p<0.02$). Systolic blood pressure decreased from 74±13 to 66±7 mmHg ($p<0.01$) and diastolic blood pressure decreased from 45±11 to 38±7 mmHg ($p<0.01$). KCl and NaCl supplementation could only be marginally decreased. No improvement was observed in the control of the metabolic alkalosis. The urinary calcium/creatinine ratio increased rapidly to 2.55–4.24 mM/mM after initiation of NaCl supplementation.

At one year of age, the follow-up renal ultrasound did not show any evidence of nephrocalcinosis. Body weight was −4.2 SD and height was −4.6 SD. Axial hypotonia had improved considerably. Fluid requirement was approximately 240 ml/kg/day. Serum creatinine was 71 μmol/L (estimated GFR=35 ml/min/1.73 m^2, according to the Schwartz formula).

At the last follow-up at the age of 26 months, the patient had persistent chronic renal failure (estimated GFR=56 ml/min/1.73 m^2, according to the Schwartz formula). Somatic growth had improved considerably with continuous nocturnal enteral feeding and treatment with recombinant human growth hormone. Both weight and height have reached the third percentile. Proteinuria and hematuria were both absent. Blood pressure was 84/52 mmHg.

B. Patient 2 was born at 37 weeks of gestation with a birth weight of 2.520 kg after a pregnancy complicated by severe polyhydramnios. The postnatal physical examination was remarkable for prominent axial hypotonia. At three months of age the child was admitted to a local hospital for severe failure to thrive and was found to have hypokalemia (2.9–3.5 mmol/L), metabolic alkalosis (HCO_3 33.5 mmol/L, BE +8.5 mmol/L), mild hyperreninemia (1.80 pmol/L) and normal aldosterone plasma level (0.454 nmol/L). FeNa was 2.1% and FeCl was 10.2%. Renal ultrasound showed diffuse renal hyperechogenicity, but no signs of nephrocalcinosis. Urinary calcium/creatinine excretion was normal (1.50 mM/mM). Arterial blood pressure was 68–57/42–25 mmHg. Hearing tests at three months of age demonstrated severe hypoacusia with an absence of evoked response to stimulation at 120 dB.

Treatment has included indomethacin (2.8 mg/kg/day), and KCl and NaCl supplementation (both at a dosage of 3.5 mmol/kg/day). Indomethacin treatment was not followed by any significant improvement in the clinical picture. In particular, no significant improvement in growth velocity was obtained until continuous enteral feeding was started at the age of eight months, resulting in a weight gain of 900 g in five weeks.

At the last follow-up at 19 months of age, the patient weight and height were both at −3.5 SD. Serum creatinine was normalized 42.4 μmol/L (estimated GFR 81 ml/min/1.73 m^2 according to the Schwartz formula), after having always been just below the lower limit for the age for the first 18 months of life. The patients never had proteinuria or hematuria. Serum potassium levels continue to be quite low (2.5 mmol/L) despite therapy. A recent follow-up ultrasound did not show signs of nephrocalcinosis.

Diuretic testing

In view of the large urinary electrolyte losses of patient 1 and of his unresponsiveness to conventional pharmacological treatment, we have hypothesized that inhibition of NaCl reabsorption was not restricted to the thick ascending limb of Henle. Therefore, renal response to furosemide and to hydrochlorothiazide was tested (Fig. 1b). As shown, no response was obtained with these drugs. In comparison, the results of three tests performed previously are reported. These tests were performed in older patients (age range 10–14 years) presenting with a difficult differential diagnosis between Gitelman syndrome and type III BS. Two of these patients had a good response to hydrochlorothiazide but none to furosemide, suggesting a diagnosis of BS, whereas one patient had the opposite pattern of response, suggesting a diagnosis of Gitelman syndrome.

Discussion

We have reported on two new cases of neonatal type IV Bartter syndrome. These cases represent a minority of our total cases of BS (approximately 2%). Both patients demonstrated a typical clinical history of neonatal type IV BS [6]. Pregnancy was complicated by severe polyhydramnios and the post-natal course was characterized by massive renal salt and water losses, poor response to prostaglandins inhibitors, severe hypotonia and poor growth.

The *BSND* gene mutation of patient 2 (3G>A) has been reported previously [2]. It is expected to cause a complete loss of function, as it abolishes the start codon for initiation of translation. The mutation of patient 1 (c.420+5G>C) is located in intron 1, five bases into the natural splicing site. The functional consequences of this substitution cannot be indisputably proven. Nonetheless, the typical clinical picture of type IV BS, the homozygosity of the mutation, the evidence that it does not correspond to a polymorphic change and the theoretical calculation predicting severe weakening of the binding site strength strongly suggest that this mutation is likely to severely alter the Barttin protein function.

Mutations in the *BSND* gene or deletions encompassing the ClCNKB and ClCNKA genes have been reported to be associated with progression of chronic renal failure, which is an uncommon complication for other types of BS [2, 9, 10]. Other authors have not confirmed these findings [6]. It has been proposed that decline in renal function may also be secondary to the nephrotoxicity of nonsteroidal anti-inflammatory agents, in particular indomethacin [11]. A recent report, however, indicates that prostaglandin inhibitors are generally tolerated well by patients with hereditary hypokalemic salt-losing tubulopathies, without evidence of impaired renal function after 10–15 years of treatment [12].

Whereas renal function was normalized at 18 months of age in patient 2, after it had constantly been just below the lower limit of normal, patient 1 demonstrated persistent chronic renal failure at her last follow-up at 26 months of age. In our view, in patient 1 it is not possible to distinguish between renal function impairment secondary to combined captopril and indomethacin therapy and increased serum creatinine secondary to structural renal changes related to the BS. As in other type IV BS cases, these two patients were remarkable for the severity of their axial hypotonia and for the magnitude of their renal salt wasting.

As previously reported, tubular dysfunction was not improved substantially by indomethacin and/or nimesulide treatment [10, 11]. Lack of response to indomethacin could not be attributed to decreased intestinal reabsorption in patient 1, as it was not improved by i.v. administration of the drug.

Also, higher dosages or a combination of both agents did not produce any substantial benefits. Accordingly, Reinalter et al. have previously documented a lack of a decrease in urinary prostaglandin excretion after indomethacin treatment in patients with type IV BS [12]. Hypothetically, chloride fluxes, which activate the *macula densa* and thereby modulate COX2 activity and renin secretion [13], may require normal Barttin expression.

Patient 1 improved only after a combined therapy with indomethacin and captopril, an approach which has been

used to control severe congenital nephrosis [11] and which may have exerted its beneficial effects primarily by decreasing the glomerular filtration rate.

The results of the diuretic tests may provide additional insight into the pathophysiology of type IV BS. Unlike the ClC-Kb channel (mutated in type III BS), the expression of the Barttin protein, which acts as a functional subunit of ClC-Kb, is not restricted to the thick ascending limb of Henle [14]. Accordingly, diuretic tests in patient 1 showed an absence of response to both furosemide and hydrochlorothiazide. Inhibition of NaCl reabsorption therefore also appears to extend to distal segments of the nephron, which may explains the magnitude of tubular NaCl losses in type IV BS. In addition, these results provide a reasonable explanation for the lack of substantial hypercalciuria and protection against the development of overt nephrocalcinosis in these patients. In fact, inhibition of NaCl reabsorption in classic BS engenders hypercalciuria, whereas decreased NaCl reabsorption in the distal convoluted tubule is associated with hypocalciuria in Gitelman syndrome or after thiazide treatment [15]. In type IV BS these opposite effects may coexist, thus limiting the magnitude of urine calcium excretion despite massive urinary NaCl losses.

It is important to note, however, that the results reported for furosemide and hydrochlorothiazide tests were obtained from only one patient. Therefore, although they appear to be coherent with the known pattern of expression of the Barttin protein in the renal tubule, they should be interpreted with caution and need to be confirmed by other investigators.

References

1. Konrad M, Weber S (2003) Recent advances in molecular genetics of hereditary magnesium-losing disorders. J Am Soc Nephrol 14:249–260
2. Birkenhager R, Otto E, Schurmann MJ, Vollmer M, Ruf EM, Maier-Lutz I, Beekmann F, Fekete A, Omran H, Feldmann D, Milford DV, Jeck N, Konrad M, Landau D, Knoers NV, Antignac C, Sudbrak R, Kispert A, Hildebrandt F (2001) Mutation of *BSND* causes Bartter syndrome with sensorineural deafness and kidney failure. Nat Genet 29:310–314
3. Brennan TM, Landau D, Shalev H, Lamb F, Schutte BC, Walder RY, Mark AL, Carmi R, Sheffield VC (1998) Linkage of infantile Bartter syndrome with sensorineural deafness to chromosome 1p. Am J Hum Genet 62:355–361
4. Vollmer M, Jeck N, Lemmink HH, Vargas R, Feldmann D, Konrad M, Beekmann F, van Den Heuvel LP, Deschenes G, Guay-Woodford LM, Antignac C, Seyberth HW, Hildebrandt F, Knoers NV (2000) Antenatal Bartter syndrome with sensorineural deafness: refinement of the locus on chromosome 1p31. Nephrol Dial Transplant 15:970–974
5. Estevez R, Boettger T, Stein V, Birkenhager R, Otto E, Hildebrandt F, Jentsch TJ (2001) Barttin is a Cl^- channel beta-subunit crucial for renal Cl^- reabsorption and inner ear K^+ secretion. Nature 414:558–561
6. Landau D, Shalev H, Ohaly M, Carmi R (1995) Infantile variant of Bartter syndrome and sensorineural deafness: a new autosomal recessive disorder. Am J Med Genet 59:454–459
7. Colussi G, Rombola G, Brunati C, De Ferrari ME (1997) Abnormal reabsorption of Na^+/Cl^- by the thiazide-inhibitable transporter of the distal convoluted tubule in Gitelman's syndrome. Am J Nephrol 17:103–111
8. Nusing RM, Reinalter SC, Peters M, Komhoff M, Seyberth HW (2001) Pathogenetic role of cycloxygenase-2 in hyperprostaglandin E sindrome/antenatal nBartter sindrome: therapeutic use of the cycloxygenase-2 inhibitor nimesulide. Clin Pharmacol Ther 70:384–390
9. Schlingmann KP, Konrad M, Jeck N, Waldegger P, Reinalter SC, Holder M, Seyberth HW, Waldegger S (2004) Salt wasting and deafness resulting from mutations in two chloride channels. N Engl J Med 25:1314–1319
10. Shalev H, Ohali M, Kachko L, Landau D (2003) The neonatal variant of Bartter syndrome and deafness: preservation of renal function. Pediatrics 112:628–633
11. Mendoza SA (1988) Nephrotoxic drugs. Pediatr Nephrol 2:466–476
12. Reinalter SC, Grone HJ, Konrad M, Seyberth HW, Klaus G (2001) Evaluation of long-term treatment with indomethacin in hereditary hypokalemic salt-losing tubulopathies. J Pediatr 139:398–406
13. Harris RC, Breyer MD (2001) Physiological regulation of cyclooxygenase-2 in the kidney. Am J Physiol Renal Physiol 281:F1–F11
14. Waldegger S, Jeck N, Barth P, Peters M, Vitzthum H, Wolf K, Kurtz A, Konrad M, Seyberth HW (2002) Barttin increases surface expression and changes current properties of ClC-K channels. Eur J Physiol 444:411–418
15. Reinalter SC, Jeck N, Peters M, Seyberth HW (2004) Pharmacotyping of hypokalaemic salt-losing tubular disorders. Acta Physiol Scand 181:513–521