Eur J Pediatr (2011) 170:1209–1211
DOI 10.1007/s00431-011-1464-z

SHORT REPORT

Bartter syndrome in two sisters with a novel mutation of the *CLCNKB* gene, one with deafness

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Received: 20 January 2011 / Accepted: 22 March 2011 / Published online: 9 April 2011

Abstract This article describes two sisters with type III Bartter syndrome (BS) due to a novel missense variant of the *CLCNKB* gene. The phenotypic expression of the disease was very different in these two siblings. In one sister, the disease followed a very severe course, especially in the neonatal period and as a toddler. Both the classic symptoms and the biochemical features of the syndrome were striking. In addition, she presented with sensorineural deafness, a complication yet unreported in this subtype of BS In contrast, the least affected sister was symptom free and the biochemical features of the disease although present remained discrete throughout the prolonged follow-up. It is suggested that such a difference in the phenotypic expression of the disease is possibly secondary to the modifier effect of a gene and/or results from environmental factor(s).

Keywords Bartter syndrome · Gene mutation · Deafness · *CLCNKB* gene · Phenotype

Introduction

Bartter syndrome (BS) is a heterogeneous disorder characterized by defects in distal tubular sodium and chloride reabsorption occurring in the thick ascending limb of the loop of Henle leading to excessive sodium, chloride, and potassium urinary excretion. These patients typically present with hypokalemia, hypochloremia, metabolic alkalosis, and hyperreninemia with normal blood pressure [7]. There are two distinct presentations of BS; antenatal BS generally more severe, associated with maternal polyhydramnios and "classical" BS which is a milder form of the disease with later onset of signs and symptoms [13].

BS has been divided into four subtypes [4, 14]. Types I and II are caused by loss of functions in the sodium–potassium–chloride transporter and in the potassium channel, respectively. Type III is associated with a mutation in the *CLCNKB* gene encoding for the chloride channel. Finally, type IV is related to mutations of the *BSND* gene encoding for barttin, a protein subunit of the CIC-Kb and CIC-Ka channels, often associated with sensorineural deafness.

The purpose of this report is to describe two sisters with type III BS. The interest lies in the fact that they have the same and yet unreported mutation of the *CLCNICB* gene and a different phenotypic expression, the one more severely affected being burdened by sensorineural deafness.

Case report

Patient 1

The first sister born at term without a history of maternal polyhydramnios from non-consanguineous French–Canadian parents was transferred to our institution, a university hospital specialized in pediatric care, for severe dehydration secondary to a prolonged bout of diarrhea. Once her acute fluid and electrolyte disturbances had been corrected, it was noticed that she remained severely hypokalemic (2.3 mm/L), hypochlore-

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mic, and in a state of chronic metabolic alkalosis. (pH 7.54, CO_2 34.1 mm/L). Both plasma renin activity at 119 ng/L/S (*N*<6 ng/L/S) and plasma aldosterone at 6,370 pmol/L (*N*< 2,100 pmol/L at 2 years of age) were extremely elevated, findings consistent with the diagnosis of BS. Serum magnesium was always normal (above 0.75 mm/L). She was treated with potassium supplementation and indomethacin. On follow-up, she had to be hospitalized repeatedly for dehydration secondary to vomiting and/or diarrhea during her preschool years. A 24-h urine collection done at age 18 years revealed the following information: volume 2,6 L, sodium 22 mmol/L, potassium 48 mmol/L, chloride 40 mmol/L, and calcium 1.52 mmol/L. She remained normotensive (115/65 mmHg) over all those years. Ultrasound examinations showed no evidence of nephrocalcinosis. She had her menarche at age 13 years and grew to reach a normal adult height of 165 cm (target height 158.5 cm±6.5). Both her parents were of rather short stature, 152 cm for the mother and 165 cm for the father. Her body weight was 90 kg.

The parents started noticing that she was hard of hearing around the age of 12. An audiogram was then performed and she was found to have sensorineural deafness with a loss of 60–70 dB from 250 to 8 kHz in both ears. She has been using hearing aids since that time. Her renal function has remained normal, her endogenous creatinine clearance being estimated at 140 ml/min/1.73 m^2 and her serum creatinine steadied at 50 μm/L.

Patient 2

The other sister, eldest of the two by 5 years was brought to medical attention only once the diagnosis of BS had been established in her sibling. She was 7 years old when blood was first drawn for a serum potassium determination. Although she had gone through early childhood without any symptoms of BS, even in hindsight, her serum potassium was extremely low at 2.1 mm/L. She had the biochemical features of BS including metabolic alkalosis, hypochloremia, and hyperreninemia. Plasma renin activity was 14 ng/L/S but plasma aldosterone never exceeded the upper limits of normal concentration (*N*<1,200 pmol/L at age 7). Serum magnesium levels were always within the normal range (0.75–0.82 mm/L). A 24-h urine collection gave the following information: volume 2.4 L, sodium 22 mmol/L, potassium 45 mmol/L, chloride 38 mmol/L, and calcium 1.44 mmol/L. She was normotensive (118/65 mmHg) throughout her follow-up and her kidneys devoid of calcinosis. She attained her menarche at age 12 years. Renal function is still normal (serum creatinine 55 μm/L) at her present age of 24 and she reached a normal adult height of 157 cm and a body weight of 87 kg. Although she never complained of hearing problems, an audiogram was performed at age 20 years and found to be normal. It is noteworthy that she could be maintained symptom free taking potassium supplements only. Finally, maternal polyhydramnios was not present at birth as was the case with her sister.

Verbal permission to use clinical information concerning these two patients was obtained from the parents and the two sisters themselves.

Methods

We first excluded homozygous deletions of *CLCNKB* in patient 1 by screening three long-range PCR products covering exons 1 to 2, exons 6 to 9, and exons 17 to 19 [12]. We also screened her for mutations in all the exons and splice functions for barttin (*BSND*) but did not find any pathogenic variant. We then screened her for mutations in all 19 exons and splice junctions from genomic DNA [6, 9, 12] and analyzed the segregation pattern of any sequence variants of interest in both sisters and their parents.

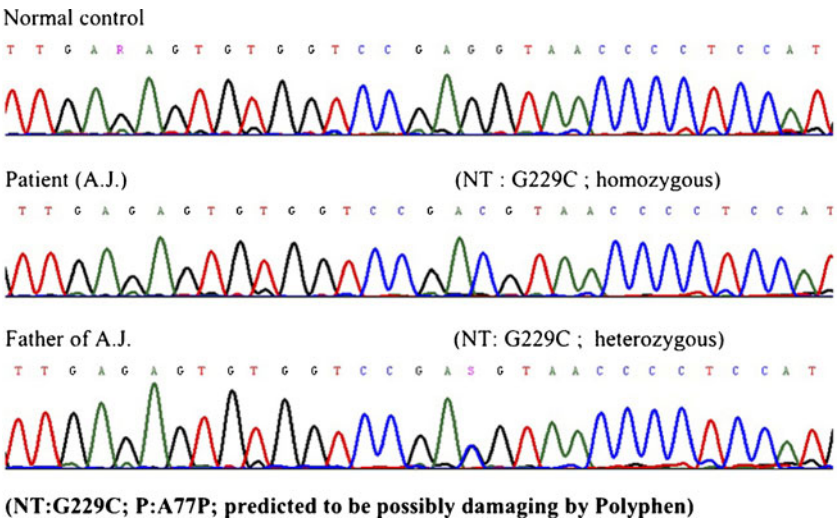

Fig. 1 Sequence tracings of a novel missense variant in exon 2 (nt:G229C;p:A77P) of *CLCNKB* was identified as homozygous in both sisters and as heterozygous in both parents (confirmed by digestion with BstEII). This variant was not detected in more than 80 Caucasian control subjects and was predicted to be possibly damaged by Polyphen (http://genetics.bwh.harvard.edu/pph/)

All the primers and PCR conditions used are available from the authors upon request.

Genetic investigation

Of 11 variations of *CLCNKB* we found in patient 1, most were reported polymorphisms, but one novel missense variant in exon 2 (nt:G229C; p:A77P) was identified as homozygous in both sisters and as heterozygous in both parents. This variant was not detected in >80 Caucasian control subjects and was predicted to be possibly damaged by Polyphen (http://genetics.bwh.harvard.edu/pph/). The corresponding sequence tracings in a normal control subject, patient 1, and her father are shown in Fig. 1.

Discussion

Although not affected at the same degree, these two sisters both have type III BS secondary to a novel missense mutation of the *CLCNKB* gene. As previously described, these patients classically had very severe hypokalemia, no nephrocalcinosis, and achieved normal adult height [1]. They kept a normal renal function as most patients with type III BS although there seems to be exceptions [8].

The difference in the phenotypic expression of the disease in these two sisters is worth emphasizing. Patient 1 presented with a much more severe clinical course, requiring frequent hospitalizations, intake of indomethacin, and eventually developing sensorineural deafness. In addition, she had an eightfold higher plasma renin activity compared to her sister whose plasma aldosterone level was contrastingly normal. The milder course in patient 2 is akin to that seen in Gitelman syndrome [5], but this diagnosis is highly unlikely since the magnesium levels were always normal, there was no hypocalciuria, and she shared the same *CLCNKB* mutations as her sister with a clinical course so characteristic of severe BS [13]. Such a dramatic difference in both clinical and biochemical expression of the syndrome between the two sisters who share the same mutations suggests a modifier effect from genetic and/or environmental factors as it has been often reported in other human diseases such as polycystic kidney disease [2].

Sensorineural deafness is usually taken to be characteristic of BS type IV [11]. Patients with this subtype tend to present with deafness at a very early age and are prone to develop renal failure [8]. The product of the affected gene called barttin is an essential beta-subunit for the chloride channel [3]. It co-localizes with the two chloride channels (CIC-Ka and CIC-KB) in the basolateral membrane of the thick ascending limb of the loop of Henle and in the potassium-secreting dark cells of the inner ear.

In our patients, barttin showed no abnormality. Barttin and both CLC-NKA and CLC-NKB are believed to interact in increasing the chloride ion flux through the channels [10]. It is tempting to surmise that the mutation observed in the *CLCNKB* here could have impacted the function of the endocochlear potential causing sensorineural deafness in the most affected sister. Although it cannot be excluded that in our patient the simultaneous occurrence of BS and deafness was only a chance association, it can be ascertained that this was a phenotypic expression of this new *CLCNKB* gene mutation. In view of our finding, it might be worthwhile performing an audiogram to further document the frequency of this complication in BS subtype III.

Conflicts of interest The authors have no relationship with the organization that sponsored the research.

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