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Exceptional Case

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Paradoxical hypertension and salt wasting in Type II Bartter syndrome

Winnie Kwai-yu Chan[1], Ka Fai To[2], Joanna H. M. Tong[2] and Chi Wai Law[1]

[1]Department of Paediatrics, Queen Elizabeth Hospital, Hong Kong SAR, China and [2]Department of Anatomical and Cellular Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong SAR, China

Correspondence and offprint requests to: Winnie Kwai-yu Chan; E-mail: chankyw@ha.org.hk

Abstract
Ante/neonatal Bartter syndrome (BS) is a rare hereditary disorder. It is characterized by renal salt wasting, hypokalaemic metabolic alkalosis, high renin and aldosterone but normal blood pressure. We report a low birth weight newborn baby who presented with repeated apnoea shortly after birth as well as hyponatraemia, hypochloraemia, hyperkalaemia and metabolic acidosis. Her biochemical features mimicked pseudohypoaldosteronism but with initial hypertension, which had not been described in BS. Her subsequent genetic study confirmed two novel heterozygous mutations in the Exon 5 of *KCNJ1* compatible with Type II BS.

Keywords: Bartter syndrome; Hypertension

Case report

We describe a pre-term baby girl born at 35 weeks of gestation with birth weight 2.26 kg, Apgar Score 6 at 1 min and 9 at 5 min. The parents' marriage was non-consanguious and both were Chinese. The pregnancy was complicated by polyhydramnios detected since 28 weeks of gestation. After birth, she started feeding with usual infant formula (SMT™) and passed urine nine times per day. She developed several episodes of shallow breathing and apnoea at 24 h of life. These attacks were associated with oxygen desaturation and bradycardia but responded readily to tactile stimulation. Her oral feeding was poorly tolerated. To supplement her milk feeding, she was given intravenous fluid at 150 mL/day, with sodium concentration of 25 mmol/L and intravenous antibiotics were started empirically after sepsis work-up. The blood test revealed a serum sodium of 124 mmol/L (normal: 136–145 mmol/L) and potassium 6.8 mmol/L (normal: 3.5–5.1 mmol/L), urea 6.4 mmol/L (normal: 1.4–6.8 mmol/L) and creatinine 78 μmol/L (normal: 21–75 μmol/L). She was not oedematous. Her lowest serum sodium dropped to 113 mmol/L on Day 4, chloride 86 mmol/L (normal: 95–105 mmol/L) and bicarbonate 18 mmol/L (normal: 22–29 mmol/L). Her fluid intake, urine output and body weight in the initial few days of life are summarized in Table 1. Despite severe salt wasting and a weight loss of 14% within the first week, her blood pressure measured via intra-arterial line with good tracing was high for a premature baby at around 80/50 mmHg (Figure 1). She had a high urine output which ranged from 7.4–10 mL/kg/h and urine osmolality was low at 145–237 mosmol/kg. Urine sodium excretion ranged from 47 to 91 mmol/L, with increased fractional excretion of urinary sodium to 4–11%. Her morning cortisol was 556 nmol/L (normal spot cortisol at 7–10 am: 171–538 nmol/L), 17αOH progesterone was 3.2 nmol/L (normal: 0.5–20 nmol/L), aldosterone >3330 pmol/L (normal: <444 pmol/L) and plasma renin activity was extremely high such that it was out of the usual range for measurement in our laboratory. A random spot urine calcium creatinine ratio was 3.8 mmol/mmol Cr (normal: <0.7 mmol/mmol Cr). Serum magnesium was 0.69 mmol/L (normal: 0.62–0.91 mmol/L). Cranial ultrasound (USG) on Day 4 did not show any intra-cranial lesions or cerebral oedema. USG kidney on Day 19 showed bilateral nephrocalcinosis. She was treated with an NaCl supplement. Her serum sodium increased to 132 mmol/L by Day 8 of life but her serum potassium slowly dropped to a lowest of 2.6 mmol/L on Day 10 with increased transtubular potassium gradient at 6.9. Genetic study confirmed two novel heterozygous mutations in the Exon 5 of *KCNJ1* compatible with Type II Bartter syndrome (BS) (Figure 2a and b). The baby inherited an E151K missense mutation from her father and an in-frame deletion of four amino acids (ANHT) from her mother. Her condition was stabilized with sodium supplement at 10 mmol/kg/day, potassium supplement at 3.4 mmol/kg/day and indomethacin at 1.5 mg/kg/day. Her blood pressure slowly dropped back to the 90th percentile without any treatment. Her estimated glomerular filtration rate by Schwartz formula at 2 weeks of life was 33.3 mL/min/1.73 m^2 (normal for age).

Discussion

BS describes a group of inherited salt-losing tubulopathy characterized by hypokalaemic metabolic alkalosis accompanied by normal or low blood pressure despite hyperreninaemia and hyperaldosteronism. It is not a single disorder but a syndrome that shares a set of closely related disorders with similar biochemical abnormalities. They differ in the age of onset, presenting symptoms, magnitudes of urinary potassium, prostaglandin and calcium excretions [1].

In the kidney, ~25% of the filtered NaCl is reabsorbed by the thick ascending loop of Henle (TAL); 5–10% is

Table 1. Change in body weight, oral and intravenous input, urine output and change in serum electrolytes in the initial few days of life of the affected baby[a]

Day	Body weight (kg)	Input/source of fluid input and sodium content in the intravenous fluid	Urine output (times/day) or mL/day	Serum sodium (mmol/L)	Serum potassium (mmol/L)	Urine osmolality (mosmol/L)	Urine sodium (mmol/L)	Fractional excretion of sodium (%)	TTKG
After birth	2.26	Usual milk formula SMT™	9 times	NA	NA	NA	NA	NA	NA
D2	2.03	IVF: 190 mL/day (95 mL/kg/day); Na^+ content: 50 mmol/L	10 times	124	6.8	NA	NA	NA	NA
D3	1.94	IVF: 300 mL/day (150 mL/kg/day); Na^+ content: 45 mmol/L	9 times	117	6.3	177	74	6.3	NA
D4	1.94 (a drop of 14%)	IVF: 432 mL/day; Na^+ content: 45 mmol/L; normal saline and hypertonic sodium infusion	410 mL (8.5 mL/kg/h)	119, 113	5.1	186	75	6.3	NA
D5	1.87	IVF: 450 mL/day; Na^+ content: 55 mmol/L	368 mL (8.2 mL/kg/h)	125	5.6	145	47	4	NA
D8	1.81	IVF: 450 mL/day; milk: 90 mL; Na^+ content: 110 mmol/L	436 mL (10 mL/kg/h)	132	3.8	195	89	6.7	NA
D9	1.87	IVF: 250 mL/day; milk: 210 mL/day; Na^+ content: 90 mmol/L	420 mL (9.4 mL/kg/h)	134	3.5	199	91	6.8	6.6
D16	2.07	Milk (expressed breast milk/SMT™) 55 mL $\times$ 8 = 220 mL/kg/day; sodium supplement 10 mmol/kg/day; potassium supplement at 3.4 mmol/kg/day	359 mL (7.4 mL/kg/h)	139	4.1	237	83	11	6.9

[a]Her birth weight was 2.26 kg and body length was 46 cm. With the profound drop in body weight, polyuria, low urine osmolality and high urine sodium excretion, salt-losing tubulonephropathy was the cause of her hyponatraemia. SMT™, Snow Brand Smart Baby 1 infant formula which contains 54.8 g carbohydrate (g/100 g), protein 12.3 (g/100 g), fat 21 (g/100 g), sodium 135 mg/100 g and potassium 460 mg/100 g. IVF, intravenous fluid; Na^+, sodium; NA, data not available; TTKG, transtubular potassium gradient.

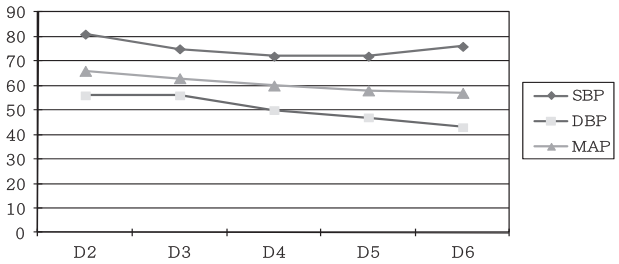

Fig. 1. Mean arterial blood pressure measured from an indwelling arterial catheter over right radial artery of the patient in the first few days of life while her blood test showed hyponatraemia, hypochloraemia and polyuria. The arterial line had a good tracing and reflected a reliable blood pressure measurement.

reabsorbed by the distal convoluted tubule (DCT) and the distal nephron segments are the final sites for regulation of sodium reabsorption. The energy for sodium transport derives from the Na-K-ATPase, which locates at the basolateral cell membrane. This Na-K-ATPase maintains a low intracellular sodium level and this in turn generates the driving force for other sodium transporters to absorb sodium at the apical membrane. These apical sodium transporters include the Na-K-2Cl co-transporter (NKCC2) at the TAL, the Na-Cl co-transporter and epithelial Na channel (ENaC) at the DCTs and connecting tubules. To maintain the normal reabsorption of urinary sodium, all cells have an exit pathway for potassium and chloride. Renal outer medullary K channel (ROMK) is the potassium channel situated at the apical membrane of TAL and distal nephrons. It helps to recycle potassium across the apical membrane [2, 3]. In the aldosterone-sensitive distal nephrons, ROMK channel is essential for potassium ion secretion in exchange for the reabsorption of sodium via the amiloride-sensitive ENaC [4]. To date, there are five types of BSs described in the literature. The gene that accounts for Type 1 BS is *SLC12A1*, which encodes the apical NKCC2. Type II BS is *KCNJ1*, which encodes the apical ROMK channel. Type III BS is *CLCNKB*, which encodes the basolateral Cl channel Kb (CIC-Kb) [3]. *BSND* is the fourth gene, which encodes barttin, a subunit for CIC-Ka and CIC-Kb, and has been identified as being responsible for a combination of antenatal BS and sensorineuronal deafness [5]. Type V BS shows various functional mutations in the calcium-sensing receptor and is associated with hypoparathyroidism [6].

Genetic study of our patient showed novel compound heterozygous mutations in the Exon 5 of *KCNJ1*. One is an in-frame deletion of 12 nucleotides causing a deletion of four amino acids (ANHT) from codon 116 to 119. Another heterozygous G-A mutation, (GAA-AAA), resulted in

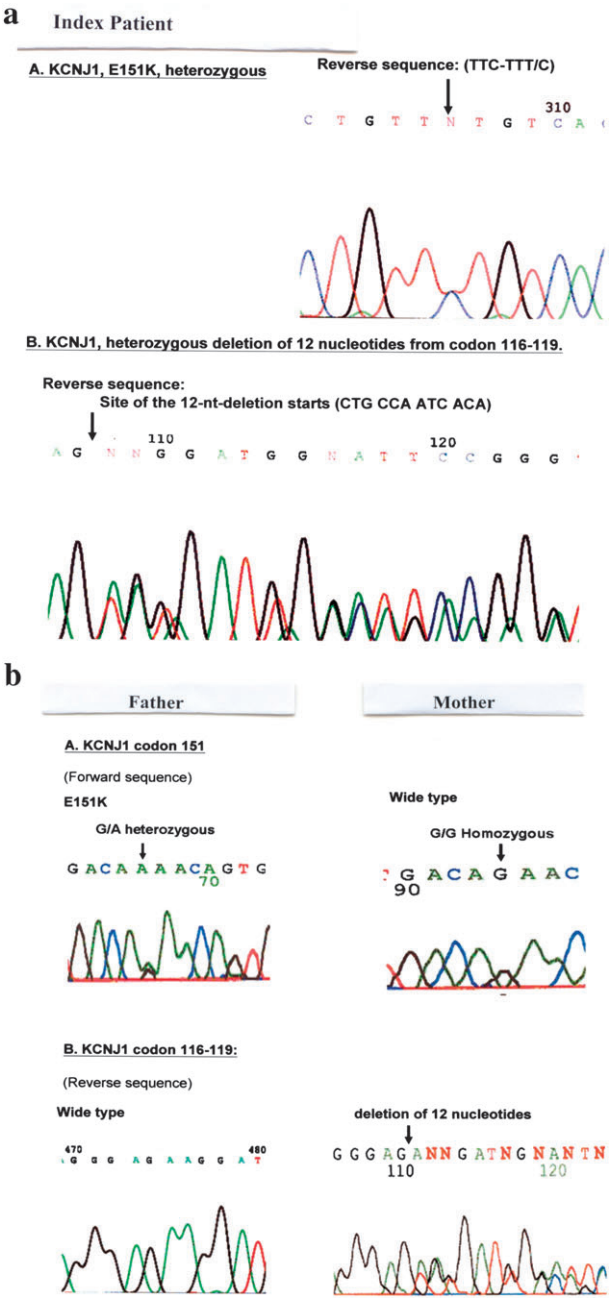

Fig. 2. (**a**) Sequencing analysis of *KCNJ1* gene: genomic DNA was used for polymerase chain reactions (PCRs) of *KCNJ1* gene Exons 1–5 and the adjacent introns. The PCR products were purified and then sequenced using the BigDye Terminator v1.1 Cycle Sequencing Kit (Appliedbiosystems) and run on an Applied Biosystems 377 Genetic analyzer. The data were collected and analysed using Applied Biosystems sequencing analysis software. The heterozygous alleles were resolved by cloning the PCR products into TOPO vectors, sequencing of multiple clones were performed. Results of genetic study of the index patient confirmed two novel heterozygous mutations in the Exon 5 of *KCNJ1*. One heterozygous G-A mutation, was identified in Exon 5 of *KCNJ1* (GAA-AAA), resulted in amino acid change from glutamic acid to lysine at codon 151 (E151K), Glu151Lys. Another heterozygous in-frame deletion of 12 nucleotides, which caused in-frame deletion of four amino acids (ANHT) from codon 116 to 119. These two mutations were located in the channel core regions and were highly suggestive of altering the channel properties resulting in BS in the affected patient. (**b**) Mother: heterozygous in-frame deletion of 12 nucelotides resulting in deletion of four amino acids (ANHT) from codon 116 to 119 was identified. Wild-type sequence was at codon 151. Because of such large in-frame deletion of four amino acids, this mutation is considered to be deleterious. Father: a heterozygous mutation at codon 151 was found (GAA-AAA) and resulted in amino acid change from glutamic acid to lysine (E151K). Wild-type sequence was at codon 116–119. In E151K missense mutation, among mammal species, the amino acid at codon 151 is highly conserved with either E or D and both are negatively charged residues. The mutation changes it to a positively charged residue. It is very likely to be deleterious. Population screening showed that the mutation E151K was not found in the screening of 160 chromosomes in the population. Such observations indicated that E151K is not a polymorphism in our population and supports that this is a disease-related mutation.

amino acid change from glutamic acid to lysine at codon 151 (E151K). It has been reported that mutations that are located near the transmembrane domains or the core region (amino acids 84–180) completely abolish functional expression [7, 8]. The in-frame deletion of codon 116–119 (ANHT) completely abolished the only N-glycosylation consensus site in the *KCNJ1* protein (amino acids 117–119), which is critical in stabilizing the open state of the channel. Experimental abolishment of glucosylation by point mutation at codon 117 or by treatment with tunicamycin greatly reduced whole cell currents [9]. Therefore, the novel in-frame deletion we found was predicted to alter channel kinetics by abolishing the glycosylation of *KCNJ1*. E151K occurred at the highly conserved motif, which is homologous to the pore-forming H5 region of voltage-gated potassium channels. This mutation might lead to defective potassium conductance due to conformational changes of the pore-forming domains or a disturbed assembly. E151K was not found in the screening of 160 chromosomes in the population, indicating that E151K is not a polymorphism in our population and supporting that it is disease-related. In silico analysis by SIFT (sift.jcvi.org) indicated that substitution at position 151 from E to K was predicted to be DAMAGING with a score of 0.00 (median sequence information: 2.72, No. of sequences represented: 116). The species conservation analysis showed that the glutamic acid (E) at codon 151 is highly conserved among mammals, signifying the importance of E in maintaining proper protein function. This information provided further evidence on the novel mutation E151K is pathogenic.

Our patient presented shortly after birth with severe hyponatraemia due to renal salt wasting. Adrenal insufficiency, particularly salt-losing congenital adrenal hyperplasia, was a possible differential diagnosis. However, the normal cortisol and 17αOH progesterone level excluded the diagnosis. The highly elevated renin–aldosterone and initial hyperkalaemia also confused the diagnosis of pseudohypoaldosteronism. In fact, the apparent feature of pseudohypoaldosteronism had been well reported in patient with neonatal BS [10]. The early nephrocalcinosis and subsequent development of hypokalaemia, however, supported the diagnosis of neonatal BS. Both Type I and Type II BS could present as severe life-threatening salt-losing polyuria with iso- or hyposthenuria in the neonatal period. The presence of transient hyperkalaemia would favour a ROMK defect as ROMK is present in the distal tubules and is essential for the final regulation of net potassium excretion.

Another unique feature observed in our patient was the high blood pressure at presentation despite severe sodium wasting, and appeared contradicting the diagnosis of BS. Hypertension in neonatal BS has not been reported in the literature and in fact, normal or low blood pressure is one of the cardinal features in BS. Defining normal blood pressure in premature infants is a complex task, yet there is normal blood pressure data for infants and preemies [11]. The transient hypertension observed in our patient was genuine as blood pressure was continuously measured

by an intra-arterial catheter with good tracing which is the gold standard for blood pressure measurement. Our patient lost weight with polyuria and was not oedematous. Her brain ultrasound did not show any intra-cranial lesion or cerebral oedema. Hence, her high blood pressure likely resulted from the increase in peripheral vascular tone and resistance. In response to salt depletion and hypovolaemia, production of renin and angiotensin is increased. Angiotensin is converted to angiotensin II, which is a potent vasoconstrictor by itself. It also stimulates the production of aldosterone to decrease urinary fluid and electrolytes loss from the body. Apart from the renin–aldosterone axis, acute hypovolaemia also triggers various sympathetic compensatory responses. These activated sympathetic discharges can act directly on the vascular smooth muscle cells and indirectly via other hormones, like catecholamines, to increase the blood pressure. We have to be caution with the hypertension detected in the presence of severe hyponatraemia. Early features of hyponatraemic encephalocepathy like headache, vomiting and altered consciousness are difficult to detect in neonates. Seizures, systemic hypertension and apnoea could represent advance hyponatraemic encephalopathy [12].

In recent years, the feature of normal to low blood pressure with a high renin–angiotensin–aldosterone secretion in BS is a focus of study. It acts as human modes for exploration of the mechanism responsible for maintenance and controlling of vascular tone and helps to explain the heritability of blood pressure variation in the general population. Calo L *et al.* [13] reported the multiple actions of angiotensin II which are mediated via specific and complex intracellular signalling pathways. These signals result in vascular smooth muscle contraction and are counterbalanced by the vasodilatory activity of the nitric oxide (NO) system. Patients with BS were shown to have reduced total peripheral resistance and were hyporesponsive to the pressor action of angiotensin II. A reduced gene and protein expression of the α subunit of Gq protein and decreased downstream intracellular events were reported [14]. An increase in expression of the endothelial subunit of NO synthase messenger RNA alongside with up-regulation of the NO system was also described [15]. The high renin and aldosterone observed in our patient was compatible with 'end-organ hyporesponsiveness'. The hypertension observed might result from exaggerated responses from other compensatory mechanisms. If peripheral vascular tone was hyporesponsive to angiotensin II in BS, the vascular response to other pressor agents probably remained intact otherwise the patient should suffer from lethal hypotension.

Conclusion

Severe salt wasting could be an early presentation of antenatal BS. The presence of initial hypertension in a newborn did not exclude the diagnosis and genetic study is a valuable tool for confirmation of the disease.

Conflict of interest statement. None declared.

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