A novel mutation associated with Type III Bartter syndrome: A report of five cases

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Received June 29, 2018; Accepted February 6, 2019

DOI: 10.3892/mmr.2019.10255

Abstract. The clinical, biochemical and mutation spectra of Chinese patients with Type III Bartter syndrome (type III BS), a rare autosomal recessive disorder, were investigated. A total of five unrelated Chinese patients aged 8 months to 24 years were diagnosed with type III BS via analysis of biochemical markers, including chloride, potassium and calcium, and genetic sequencing. The levels of insulin-like growth factor-1 (IGF-1) were evaluated via ELISA and a mutation study of cultured amniocytes was conducted for prenatal diagnosis. The child patients were admitted for polydipsia, polyuria, myasthenia and developmental delay, whereas the adult patients were hospitalized for limb numbness, polydipsia and polyuria. Nine variants in the chloride voltage-gated channel Kb (*CLCNKB*) gene were detected, including eight sequence variants and one whole *CLCNKB* gene deletion. One sequence variant (c.1967T>C) was novel, whereas the remaining variants (c.595G>T, c.908A>C, c.1004T>C, c.1312C>T, c.1334_1335delCT and c.1718C>A) and the whole gene deletion had been previously reported. The whole gene deletion was frequently observed in patients with early-onset type III BS in the present study. Two patients showed IGF-1 deficiency with normal growth hormone level. All patients were treated with potassium supplementation and indometacin. The mother of one patient underwent amniocentesis during her second pregnancy; the fetus was not affected by type III BS based on screening for sequence variants, and normal development and blood electrolyte analysis following birth confirmed the diagnosis. In conclusion, five cases of type III BS in patients from mainland China were reported. Large deletions were frequently detected, particularly in early-onset patients; isolated IGF-1 deficiency was found, one novel sequence variant was identified. Prenatal diagnosis was successfully established using genetic analysis of cultured amniocytes, and may facilitate the prevention of congenital defect of type III BS in the next pregnancy.

Introduction

Type III Bartter syndrome (Type III BS; Online Mendelian Inheritance in Man no. 607364), also termed classic Bartter syndrome, is a rare autosomal recessive disorder induced by pathogenic sequence variants in the chloride voltage_gated channel Kb (*CLCNKB)* gene (1) that affect salt reabsorption in the thick ascending loop of Henle. BS was first reported in 1962 (2). The prevalence of the disorder in Costa Rica, Kuwait and Sweden was estimated to be 12 (3), 17 (4) and 1.2 individuals per 1,000,000 (5), respectively. The prevalence of the disorder in the Chinese population is unknown.

The *CLCNKB* gene encodes chloride channel KB (ClC-KB), a member of the ClC chloride channel family that serves as a component of ClC-K/Barttin ClC-K-type accessory β subunit (BSND) heteromers and is involved in sodium reabsorption (6). Defects in the ClC-KB protein results in the suppression of sodium reabsorption, renin-angiotensin-aldosterone system (RAAS) dysfunction and aldosterone secretion (1,7). The clinical manifestations of type III BS are heterogeneous, including developmental retardation, polyuria, polydipsia, cramps, dehydration, constipation, renal dysfunction and in certain cases, sudden death (8); however, the mechanisms underlying the phenotypic heterogeneity of the disorder remain unknown.

Plasma biomarkers and genetic mutations are used in the diagnosis of type III BS. In addition, RAA levels in blood are a biochemical diagnosis method (1,7). Genetic testing has revealed ~100 sequence variants in the *CLCNKB* gene (http://www.hgmd.cf.ac.uk/ac/index.php). p.A204T was described as the founder mutation of Type III BS in Spain (9).

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Key words: Type III Bartter syndrome, chloride voltage-gated channel Kb gene, insulin-like growth factor-1

A previous study observed that the deletion of the *CLCNKB* gene is a frequent pathogenic variation in the Chinese population (10).

Type III BS is the most common among the Bartter syndrome group of kidney disorders globally; however, only a small number of cases have been reported in mainland China (10), as prenatal diagnosis is not common in the country. The present study characterized the clinical, biochemical and mutation spectra of five Chinese patients with type III BS.

Materials and methods

Patients. A total of 5 patients aged 8 months to 24 years (2 male children, 1 female child and 2 adult males) from 5 unrelated Chinese families were firstly diagnosed with type III BS at the Department of Pediatrics, Peking University First Hospital (Beijing, China), and the Department of Nephrology, General Hospital of Tianjin Medical University (Tianjin, China) between January 2011 and October 2017. The clinical symptoms of type III BS developed between the ages of 3 months and 5 years. The patients were admitted with presentations of polydipsia, polyuria, low weight, myasthenia, cramps and fatigue (Table I). The parents of the patients were all healthy and nonconsanguineous. The mother of Patient 2 was subsequently admitted at 20 weeks of pregnancy seeking genetic counseling and prenatal screening for type III BS. Additionally, a total of 100 male and 100 female patients were recruited between December 2014 and January 2016 in The Peking University First Hospital and General Hospital of Tianjin Medical University, were recruited as normal controls for insulin-like growth factor-1 (IGF-1) analysis; the individuals were divided into four age groups (1-3, 8-10, 18-21 a nd 22-24 years). The study was conducted in accordance with the Code of Ethics of the World Medical Association and the Declaration of Helsinki (11). The present study was approved by the Institutional Review Board of the General Hospital of Tianjin Medical University, Institute of Hematology and Blood Diseases Hospital, and Beijing University First Hospital. Informed consent for biochemical and genetic analysis was obtained from all patients or parents of patients.

Routine tests and evaluation of IGF-1 levels. A total of 4 ml peripheral venous blood was centrifuged for 5 min at 1,000 x g and 20˚C, and laboratory tests for liver and renal function, electrolytes, glucose, ammonia, creatine kinase (UniCel DxC600; Beckman Coulter, Inc.) were performed. In total, 2 ml peripheral venous blood was centrifuged for 5 min at 1,000 x g and 4˚C, and renin and aldosterone (LIAISON XL; DiaSorin, Inc.) levels were measured. Then, 2 ml arterial blood was collected from the radial artery, and blood gas (Cobas b 123; Roche Diagnostics) test was performed according to the manufacturer's protocol. Additionally, 2 ml peripheral venous blood from each patient and healthy controls was centrifuged for 15 min at 1,800 x g and 4˚C, and immediately stored at -80˚C. Levels of IGF-1 were measured using an ELISA kit (cat. no. DG100; R&D Systems, Inc.). Moreover, 1.5 ml peripheral venous blood was collected at 0, 30, 60, 90 and 120 min after GH excitation for the GH excitation test [L-dopa stimulation test was performed in children, and insulin tolerance test (ITT) in adults]. The samples were centrifuged for 10 min at 1,000 x g and 4˚C, GH levels were measured using a DXI800 (Beckman Coulter, Inc.) and the GH peak was calculated.

Prenatal diagnosis. The mother of Patient 2 opted to undergo prenatal diagnosis of type III BS. This was the second pregnancy of the 31-year-old female. Amniocentesis was performed at 20 weeks gestational age. Amniotic fluid was collected by transabdominal amniocentesis under ultrasound guidance. The amniotic fluid was immediately analyzed.

CLCNKB gene analysis. Genomic DNA was extracted from peripheral blood lymphocytes from patients, their parents, and amniotic fluid cells using the TIANamp blood DNA kit (Tiangen Biotech Co., Ltd., Beijing, China). Whole exome sequencing was performed using SureSelectXT Human All Exon V6 (Agilent Technologies, Inc., Santa Clara, CA, USA); exons and flanking intronic regions of the variants detected by whole exome sequencing were amplified via PCR and then sequenced, as previously described (12). The results were compared with the reference sequence of *CLCNKB* (NM_000085) deposited in the UCSC genome (genome.ucsc.edu/). Sequencing data were compared with an integrated set of variants (hgmd.cf.ac.uk), genotypes and haplotypes from the 1,000 Genomes Project (1000genomes.org) to identify mutations.

Prediction of the effects of the sequence variants in the CLCNKB gene and conservation analysis. Numerous sequence alignments were performed to verify the degree of sequence conservation of p.L656 of the ClC-KB protein. The PolyPhen-2 (Polymorphism Phenotyping; version 2; genetics.bwh.harvard.edu/pph/) and MutationTaster (MutationTaster; version 2; mutationtaster.org/) programs were used to predict the effects of missense alterations on protein function. Similarly, numerous sequence alignments were obtained using the Basic Local Alignment Search Tool (BLAST+ version 2.7.1; release date, October 23, 2017; blast.ncbi.nlm.nih.gov/Blast.cgi).

Single-nucleotide polymorphism (SNP) array analysis. Microdeletion screening was performed using an SNP array platform (Affymetrix GCS 3000Dx v.2; Affymetrix; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with a GeneChip (Affymetrix CytoScan HD/750k; Affymetrix; Thermo Fisher Scientific, Inc.). Array data were then analyzed using the GenomeStudio version 2010.1, KaryoStudio version 1.2 (standard settings; Illumina, Inc., San Diego, CA, USA) and Nexus Copy Number 5.0 (BioDiscovery, Inc., El Segundo, CA, USA) (13).

Results

Clinical data and laboratory examinations. Table I presents the clinical data of patients and the results of the laboratory assays. With the exception of Patient 1, all patients were born at term. All patients were from nonconsanguineous families. The pediatric patients were admitted for polydipsia, polyuria and developmental retardation, whereas the adult patients first presented with fatigue, polydipsia and polyuria, and

Table I. Clinical and laboratory data of five Chinese patients with type III Bartter syndrome.

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Normal range
Gender	Male	Female	Male	Male	Male	
Age of onset	3 m	5 m	6 m	5 y	3 y	
Age of diagnosis	8 m	3 y	8 y	24 y	18 y	
Present age	3 y	9 y 11 m	9 y 9 m	24 y	19 y	
Symptoms and signs						
Polydipsia	-	+	+	+	+	
Polyuria	-	+	+	+	+	
Myasthenia	+	-	-	-		
Developmental retardation	+	+	+	+	+	
Cramps	-	+	-	+	-	
Fatigue	-	-	-	+	+	
Orthostatic hypotension	-	-	-	+	+	
Positive family history	-	-	-	-	-	
Laboratory findings						
pH (blood gas analysis)	7.647↑	7.48↑	7.75↑	7.55↑	7.71↑	7.34-7.45
Serum Cl^- (mmol/l)	89↓	93↓	98	96↓	85↓	98-106
Serum Na^+ (mmol/l)	132 ↓	142	139	125↓	137	135-145
Serum K^+ (mmol/l)	1.76↓	2.3↓	2.7↓	2.4↓	2.5↓	3.5-5.5
Serum Mg^{2+} (mmol/l)	0.87	0.65↓	0.97	0.89	0.77↓	0.8-1.2
CO_2 combining power (mmol/l)	33.8↑	43.1↑	29.3↑	26.6	32.1↑	22-28
Renin [ng/ml/h (recumbent)]	6.57↑	2.53↑	4.31↑	1.48↑	3.65↑	0.05-0.79
Aldosterone [ng/l (recumbent)]	204.3↑	104.6	165.43↑	135.69	216.5↑	12.0-157.5
GH peak (ng/ml) of GH excitation test	11.40 (60 min)	2.14↓ (90 min)	5.21↓ (60 min)	10.37 (60 min)	13.84 (60 min)	>10 ng/ml in children >5 ng/ml in adults
IGF-1 (ng/ml)	24-51↓	27↓	19↓	74↓	143	78-242 (1-3 y); 87-353 (8-10 y); 122-379 (18-21 y); 105-342 (22-24 y)
Outcome	Healthy	Healthy	Low weight	Hypovolemia, low weight	Low weight	

y, years; m, months.

subsequently exhibited cramps. Following diagnosis of type III BS, the patients were treated with potassium supplements and indometacin. When gastrorrhagia was detected in Patient 4, indometacin treatment was suspended. The GH excitation test results identified two patients with GH deficiency and partial GH deficiency, whereas the others were normal (in children, GH response peak <5 ng/ml indicated GH deficiency; 5-10 ng/ml indicated partial GH deficiency; >10 ng/ml indicated normal GH levels. In adults, GH response peak <5 ng/ml indicated GH deficiency; >5 ng/ml indicated normal GH levels. The patients with defects in GH levels received recombinant human somatropin injection treatment. The development and symptoms of type III BS improved in all patients following treatment; however, low weight was observed in Patients 3, 4 and 5, and postural hypotension and reduced exercise tolerance were observed in the adult patients.

Prolonged hypokalemia was detected in Patient 1 following the development of a severe cold. The patient, a male aged 3 years, had been delivered prematurely, and thus, presented with extremely low weight and a history of hypokalemia from the age of 3 months. He came to our hospital when he was 8 months old, his height (65.5 cm) and body weight (6.5 kg) were below average upon presentation; however, chronic diseases or a family history of sudden death were not reported. Biochemical analysis of the serum of the patient revealed low levels of chloride, sodium, potassium and IGF-1 (24 ng/ml), elevated blood gas pH, and high levels of CO_2 combining power, renin and aldosterone. GH excitation test was not performed because of the age and the conditions of the patient. The patient, who was diagnosed with type III BS at the age of 8 months, returned to normal development following potassium and indometacin treatment. However,

Table II. Mutations detected in the *CLCNKB* gene among five Chinese patients with type III Bartter syndrome.

Patient	Mutation at nucleotide level	Mutation type	Mutation at protein level	PolyPhen-2.0 prediction and score	MutationTaster prediction and score	Conservation	Frequency	(Refs.)
1	1p36.13 16,367,268-16, 383,421 deletion	Homozygous	N/A	N/A	N/A	N/A	2/10	The present study
2	1p36.13 16,367,268-16, 382,989 deletion	Heterozygous	N/A	N/A	N/A	N/A	1/10	The present study
	c.1967T>C	Heterozygous	p.L656P	Probably damaging (0.99)	Disease causing (0.99)	Conserved in mammals	1/10	The present study
3	1334_1335delCT	Heterozygous	S445Ffs*5	N/A	Disease Causing (0.99)	Yes	1/10	(30)
	595G>T	Heterozygous	E199Ter	N/A	Disease Causing (0.99)	Yes	1/10	(31)
4	1312C>T	Heterozygous	R438C	Probably damaging (0.999)	Disease causing (0.99)	Yes	1/10	(32)
	908A>C	Heterozygous	Q303P	Probably damaging (0.987)	Disease causing (0.99)	Conserved in mammals	1/10	(33)
5	1004T>C	Heterozygous	L335P	Probably damaging (0.999)	Disease causing (0.99)	Conserved in mammals	1/10	(34)
	1718C>A	Heterozygous	S573Y	Probably damaging (0.999)	Disease causing (0.99)	Conserved in mammals	1/10	(35)

CLCNKB, chloride voltage-gated channel Kb; N/A, not applicable.

when he was 3 years old, decreased IGF-1 (51 ng/ml) with normal GH excitation test were found.

Patients 2 and 3, a female aged 9 years and 11 months, and a male aged 9 years and 9 months, presented with a similar medical history. They were admitted to the hospital with growth retardation, Patient 2 at 3 years of age and Patient 3 at 8 years. Polydipsia and polyuria were initially detected, followed by electrolytic metabolic disturbance and RAAS dysfunction. Delayed bone age and low levels of IGF-1 were detected in the two patients. Patient 2 showed partial GH deficiency and Patient 3 exhibited GH deficiency. Patient 2 and 3 received an injection of human recombinant GH, potassium supplementation and indometacin treatment. The two patients subsequently returned to school; however, Patient 3 continued to exhibit low weight (23 kg), whereas Patient 2 met the weight criteria for normal development.

Patients 4 and 5, 24- and 19-year-old males, respectively, were admitted separately but shared a similar medical history. Initial medical complaints included fatigue, low weight, polydipsia, polyuria, discontinuous cramps and reduced exercise tolerance. Polydipsia and polyuria were first observed in Patient 4 at the age of 13 years, whereas low weight and muscle weakness were first detected in Patient 5 at the age of 7 years. Postural hypotension was identified in the two patients following admission at 24 and 18 years of age, respectively. Patient 4 was 164 cm tall and weighed 47 kg, whereas Patient 5 was 174 cm tall and weighed 57 kg. Results from the GH excitation test (insulin tolerance test) of both patients were normal. Patient 4 underwent plasma transfusion monthly for hypovolemia. After 3 months of treatment, the two patients experienced relief from polydipsia, polyuria and discontinuous cramps; however, gastrorrhagia was detected in Patient 4, following which indometacin treatment was suspended.

Laboratory examinations. All five patients exhibited markedly reduced levels of serum potassium and chloride, increased levels of renin and metabolic alkalosis compared with healthy controls (Table I). Additionally, increased levels of aldosterone, and decreased serum levels of magnesium and sodium were observed in certain patients. Compared with the healthy controls, notably reduced IGF-1 levels were observed in 4 patients (Patient 1-4), while Patient 1, 4 and 5 had normal serum levels of GH (Table I).

Molecular analysis. In addition to eight previously reported variants identified in the patients (c.1967T>C, c.595G>T, c.908A>C, c.1004T>C, c.1312C>T, c.1334_1335delCT, c.1718C>A and whole *CLCNKB* gene mutation), the novel sequence variant c.1967T>C (p.L656P) was found (Table II). Genetic variants were also detected in the parents of patients. The whole gene deletion regions were 1p36.13 (16,367,268-16,383,421) del and 1p36.13 (16,367,268-16,382,989 (Fig. 1). Previous studies usually described whole gene deletions without listing the specific regions that were deleted; thus, it remains unclear whether the observed deletions have been reported prior to the present study. Sanger sequencing was performed to analyze the c.1967T>C mutation, and a 'false positive' homozygous variant (Fig. S1) was identified, as it was confirmed to be heterozygous by SNP array test. This mutation was not detected in the 1,000 Genomes Project database.

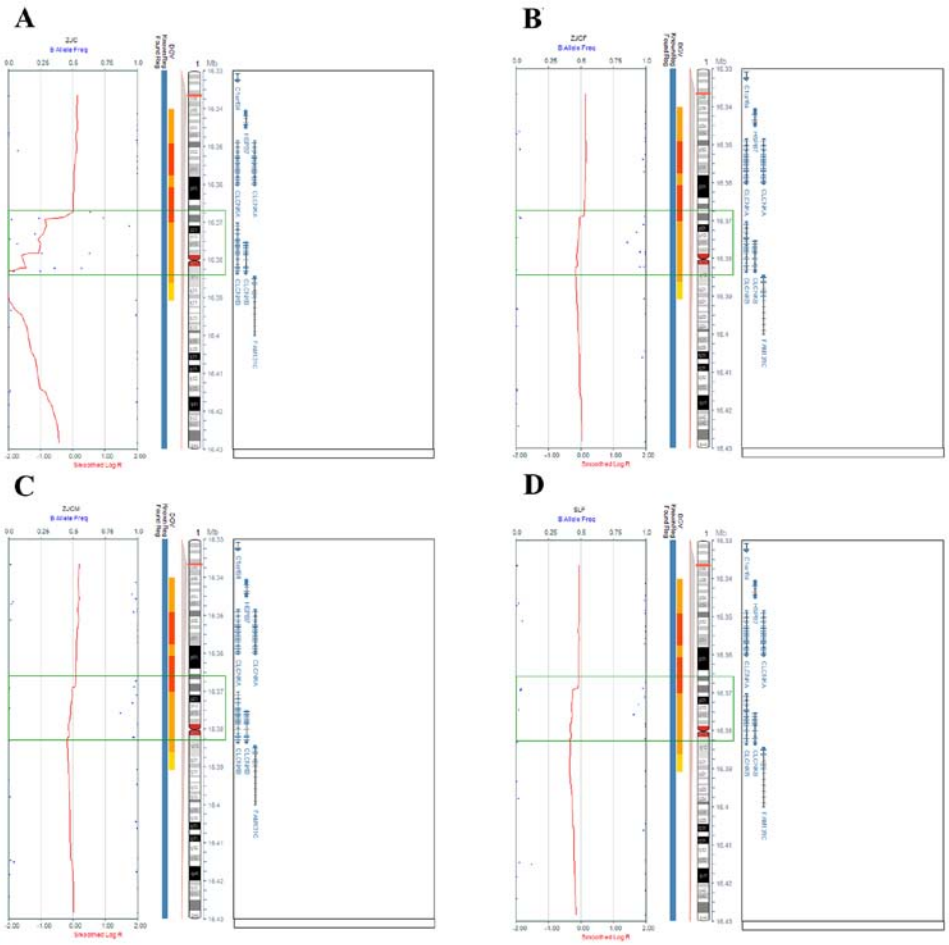

Figure 1. SNP array analysis of Family 1 and Patient 2. Scattered blue points regions and green rectangles indicate deleted regions. Homozygous and heterozygous 16.15 kb microdeletions (16,367,268-16,383,421 in chromosome 1p36.13) were detected in (A) Patient 1, and the (B) father and (C) mother, comprising the whole *CLCNKB* gene. (D) Heterozygous 15.72 kb microdeletion (from 16,367,268-16,382,989 in chromosome 1p36.13) detected in Patient 2, comprising the whole *CLCNKB* gene.

Sequence alignment of the *CLCNKB* gene revealed that the L656 amino acid is highly conserved in humans and other mammals (Table III), including *Pan troglodytes*, *Mus musculus*, *Takifugu rubripes* and *Danio rerio*, but not in *Drosophila melanogaster* and *Xenopus tropicalis*. The mutation detected in the present study was predicted to be 'probably damaging' and 'disease causing', according to PolyPhen-2.0 and MutationTaster programs, respectively.

Prenatal diagnosis. Only one heterozygous deletion was detected from amniocytes harvested from the mother of patient 2, indicating that the fetus was not affected by type III BS, due to the fact that the fetus did not share the same mutation of the proband. Normal serum electrolytic and RAAS activity level analyses were performed following delivery. At 9 months of age, the infant exhibited normal psychomotor development.

Discussion

In the present study, five cases of type III BS were reported in patients from mainland China, including symptoms, clinical findings, treatment and response. As only a small number of cases of type III BS have been reported in this population (10), the present findings may aid the evaluation of future cases in the Chinese population. Furthermore, two patients (Patient 1 and 4) with type III BS exhibited decreased serum levels of IGF-1 and normal serum GH levels. A novel mutation c.1967T>C and whole gene deletion were reported in patients with early-onset type III BS in the present study.

The *CLCNKB* gene encodes the voltage-gated chloride channel ClC-KB, which is located on chromosome lp36 (14). ClC-KB, ClC-KA and Barttin are involved in chloride absorption, and are primarily expressed in the renal thick ascending limb, distal convoluted tubules, connecting tubules and collecting tubes. Enhanced distal convoluted tubule sodium chloride reabsorption eventually leads to the loss of potassium in urine and increased hydrogen ion secretion (1). Additionally, the exchange of bicarbonate for chloride ions decreases the chloride absorption defect; therefore, bicarbonate retention and hypokalemia leads to metabolic alkalosis (1).

Renal tubular salt wasting results in the loss of water from the body. Physiologically, salt absorption in the loop of Henle results in the generation of concentrated urine. Therefore, urinary concentration and dilution defects occur in patients with type III BS (15). Defective sodium chloride reabsorption leads to an abnormal electrochemical gradient, which leads to additional calcium and magnesium reabsorption defects (1). Furthermore, hypokalemia and elevated levels of prostaglandin

Table III. Amino acid alignment of p.L656 residue of CLC-KB.

Organisms	ClC-KB sequences
Homo sapiens	LLNLHS**L**FVTS
Pan troglodytes	LLNLHS**L**FVTS
Mus musculus	LLTLQA**L**FVTS
Takifugu rubripes	LVGAKT**L**FVAD
Danio rerio	ITGEQR**L**F I T E
Drosophila melanogaster	MVGINH**A**FYVT
Xenopus tropicalis	LLGLNR**A**YVTK

The aminoacid in position p.656 is highlighted in bold. CLC-KB, chloride channel KB.

E2 (PGE2) aggravate the condition, and eventually lead to polydipsia, polyuria and hypovolemia, and low-to-normal blood pressure under the activated RAAS (16). The decrease in blood volume activates the RAAS and subsequently induces hyperaldosteronism, secondary juxtaglomerular apparatus hyperplasia and increases in the levels of renin (16). Aldosterone accelerates injury via hemodynamic alterations, cytokine expression and renal fibrosis promotion (17).

Patients with type III BS are typically asymptomatic or mildly symptomatic for 2 years after birth (1), which is why certain patients were not diagnosed until late adolescence or early adulthood. The disease presents with highly variable phenotypes, including polyuria, polydipsia, nocturia, halophilic tendencies, dehydration, vomiting, constipation and muscle weakness (1,5). Electrolyte and RAAS disorders are frequently reported, including hypokalemic metabolic alkalosis, normal or slightly increased urinary calcium with decreased serum magnesium, high PGE2 production, and increased renin and aldosterone with normal blood pressure (1). Similarly, nephrocalcinosis is frequently observed in patients with BS due to excessive loss of calcium in urine. The patients included in the present study were admitted due to hypokalemia, metabolic alkalosis and secondary hyperaldosteronism. The majority of the patients exhibited good prognosis; however, Patient 4 had certain abnormal phenotypes, including low weight, hypovolemia and orthostatic hypotension.

IGF-1, which is synthesized by the liver, is an important regulator of the proliferation, differentiation and apoptosis of cells. It serves an important role in physiological development and the pathology of various diseases and disorders, including neuroinflammation (18), heart failure (19) and hepatocellular carcinoma (20). To the best of our knowledge, although combined GH and IGF-1 deficiency were found in type III BS patients, which may be associated to hypokalemia (21), decreased serum IGF-1 with normal GH levels in type III BS have not been previously reported. Flyvbjerg *et al* (22) revealed that low potassium-induced reductions in serum IGF-1 levels and growth defects in young rats. Furthermore, angiotensin II has been hypothesized to activate nicotinamide adenine dinucleotide phosphate oxidase expression and promote reactive oxygen species-induced downregulation of IGF-1 (23). Additionally, Zaika *et al* (24) reported that IGF-1 stimulated epithelial sodium channel-mediated sodium transport in principal cells and ClC-K2 channels in intercalated cells, thus facilitating cooperative sodium and chloride reabsorption in the cortical collecting duct. IGF-1 has been observed to be an effective treatment for muscle weakness in a mouse model of spinal and bulbar muscular atrophy (25). A deficiency in IGF-1 may contribute to muscle weakness and developmental delay in type III BS patients. Therefore, it was hypothesized that low serum potassium content and high levels of angiotensin II were involved in growth retardation and decreased reabsorption function via reductions in the serum levels of IGF-1.

It has been reported that *CLCNKB* gene deletion is frequently observed in Chinese patients with type III BS (~32%) (10). However, genetic testing results are frequently misinterpreted by Sanger sequencing or next-generation sequencing due to undetectable deletions. In the present study, the homozygous c.1967T>C mutation was first observed in Patient 2 and SNP array analysis subsequently revealed a heterozygous 15.721-kb deletion, suggesting that the homozygous mutation (c.1967T>C) identified was a false positive. The deleted regions comprised a region between one point shared among all patients and different termination positions, causing the deletion of chr1 16,367,268-16,383,421 in Patient 1, and chr1 16,367,268-16,382,989 in Patient 2.

The *CLCNKB* gene deletion appeared to induce the greatest effect on early-onset patients in the present study; however, the symptoms did not notably vary from patients possessing missense mutations. In addition, whole-gene deletions have been reported in adult-onset patients (26); therefore, the genotype-phenotype association in Chinese patients remains unclear. Patients in an African American cohort possessing a homozygous *CLCNKB* gene deletion exhibited partial correction of hypokalemia and suboptimal growth even following therapy (27). *CLCNKB* mutations located in Barttin-binding sites, dimer interfaces and selectivity filters frequently induce severe functional consequences, as ClC-KB and ClC-KA require interactions with BSND to function optimally (28).

Patients with type III BS require lifelong potassium supplementation. Cyclo-oxygenase inhibitors, such as indomethacin (12), are also frequently prescribed to suppress plasma renin activity. Early diagnosis and treatment are important for the improvement of hypokalemia, hypercalciuria, developmental retardation and renal dysfunction. It was previously reported that, even with treatment, certain patients with type III BS exhibited pathological proteinuria and kidney dysfunction following a median follow-up of 14 years (29). In the present study, all patients exhibited reduced blood potassium chloride levels, however, following potassium and indometacin treatment, electrolyte disorders were markedly corrected and development notably improved, particularly in children.

In conclusion, a novel mutation associated with type III BS was reported in a Chinese patient cohort. Additionally, an IGF-1 deficiency was observed in patients. The identification of a frequent mutation (whole *CLCNKB* gene deletion) in the Chinese population may aid improvements in the genetic diagnosis of the disorder in the future. A large-scale study in China is required to investigate the mutation spectra of disease-causing genes associated with type III BS in the Chinese population.

Acknowledgements

Not applicable.

Funding

The National Natural Science Foundation of China (grant no. 81471097), The National Key Research and Development Program of China (grant no. 2017YFC1001700), and the Tianjin Research Program of Application Foundation and Advanced Technology (grant no. 15JCYBJC26200) supported the present study.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YY and DL designed the present study. YL and CW collected and analyzed the samples, and performed the experiments. JG analyzed the follow-up data. YY revised and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was conducted in accordance with the Code of Ethics of the World Medical Association and the Declaration of Helsinki. It was approved by the Institutional Review Board of the General Hospital of Tianjin Medical University, Institute of Hematology and Blood Diseases Hospital, and Beijing University First Hospital. Informed consent was obtained from all patients or parents of patients.

Patient consent for publication

All parents or legal guardians of the patients signed written informed consent forms.

Competing interests

The authors declare that they have no competing interests.

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