

A Chinese Girl with Bartter Syndrome Type III due to a Novel Mutation and/or Single Nucleotide Polymorphisms (SNPs) in CLCNKB Gene

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Abstract

Background: Bartter's syndrome is a heterogeneous disorder characterized by deficient renal reabsorption of sodium and chloride, and hypokalemic metabolic alkalosis with hyper-reninemia and hyperaldosteronemia. Bartter syndrome type III (BS type III), due to mutations in the CLCNKB gene, is highly variable. The aim of our study was to describe the clinical presentation in a Chinese girl with BS type III and to explore mutations or SNPs of CLCNKB gene in her family.

Case Presentation: The clinic data of the patient was collected. Mutations or SNPs were investigated by sequencing of the exon of CLCNKB gene. The clinic analysis confirmed the diagnosis of BS type III. The coexistence of 13 reported SNPs and 11 novel SNPs of CLCNKB gene were found in the patient and her parent. A novel heterozygous C to G transition at nucleotide 2471 in exon 20 of CLCNKB gene harbored uniquely by the patient were revealed.

Conclusion: A novel heterozygous C to G mutation at nucleotide 2471 of CLCNKB gene and some new SNPs were identified in a Chinese girl with BS type III having persistent hypokalemia. The novel mutation and SNPs make the genetic background of the patient more complicated.

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Key Words: Bartter Syndrome; Hypokalemia; Chloride Channel; Metabolic Alkalosis; Mutation

Introduction

Bartter's syndrome (BS) is a heterogeneous disorder characterized by deficient renal reabsorption of sodium and chloride, and hypokalemic metabolic alkalosis with hyper-reninemia and hyperaldosteronemia. The main pathogenesis in BS is the defect of NaCl reabsorption in the thick ascending limb of Henle's loop (TALH). Inherited BS can be divided into five subtypes according to the different inactivating mutations of genes: SLC12A1 mutation encoding the bumetanide-sensitive Na⁺-

K⁺-2Cl⁻ cotransporter (NKCC2) (type I) and KCNJ1 encoding the inward rectifying K⁺ channel (ROMK) (type II) for antenatal BS (aBS), CLCNKB encoding the kidney-specific basolateral chloride channel for cBS (type III); BSND encoding the β-subunit of the basolateral chloride channel for aBS (type IV), and gain-of-function mutations in the calcium-sensing receptors for autosomal dominant hypocalcemia associated with hypokalemia (type V)^[1].

The features of BS can be shared with Gitelman's syndromes, CLCNKB gene is expressed not only in TALH but also in distal convoluted

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tubules^[2]. BS type III has a group of autosomal-recessive inherited disorders with clinical characteristics such as renal salt wasting, hypokalemic metabolic alkalosis, elevated renin and aldosterone levels, with normal or low blood pressure. BS type III is highly variable and usually presents as a “classic” Bartter variant characterized by an onset in early childhood and less severe or absent hypercalciuria and nephrocalcinosis^[3]. In this report, we described a Chinese girl with BS type III who developed persistent hypokalemia and investigated mutations or single nucleotide polymorphisms (SNPs) of CLCNKB gene in her family.

Case Presentation

An 8^{11/12}-year-old Chinese girl was brought to the pediatric endocrinology ward because of hypokalemia found occasionally. The girl showed no progressive muscle weakness of the extremities. Her serum potassium was 2.8 mmol/l estimated two weeks ago in a local hospital. Her birth history revealed full-term by normal spontaneous delivery and normal birth weight of 3,400 g without antenatal polyhydramnios.

On admission, her height (129 cm) and body weight (25 kg) were all more than the 40th percentile. Blood pressure was 106/66 mmHg, heart rate 96 beats/min and respiratory rate 22/min. internal jugular vein was flat. There was no diminished muscle strength and no reflex over the lower extremities. Fasciculation, myoclonus

and muscular atrophy were not present. Her pure tone audiometry was normal. The remainder of the physical examination was unremarkable.

Complete blood cells counts showed that hemoglobin was 116g/l, white blood cells 5,600/ μ l and platelets 314,000/ μ l. Urinalysis revealed pH 7.5, negative protein, glucose, leukocytes, and red blood cells. The data of biochemical studies are shown in Table 1 and Table 2. The most striking findings were hypokalemia (2.5-3.0 mmol/l), mild metabolic alkalosis (HCO₃⁻ 32.6 mmol/l), normal renal function (BUN 36.4 mg/dl, Cr 3.52 mg/dl), and normal aldosterone concentration (120 pg/ml).

The trans-tubular potassium gradient (TTKG) is an index reflecting the conservation of potassium in the cortical collecting ducts of the kidneys. The following is the formula for calculating the TTKG: $TTKG = \frac{[\text{urine potassium}] \times (\text{plasma osmolality})}{[(\text{plasma potassium}) \times (\text{urine osmolality})]}$. Her TTKG (9.6) was higher, indicative of renal K⁺ wasting. Serum magnesium (Mg²⁺) was tested twice and the value was 1.82, 2.0 mg/dl respectively. Serum calcium (Ca²⁺) levels were normal, but urinary Ca²⁺ excretion was very low (urine Ca²⁺/Cr 0.01 mg/mg) (Table 1 and 2). EKG disclosed a sinus rhythm. Abdominal sonography revealed bilateral normal size kidneys and adrenal glands without nephrocalcinosis or renal stones. With the above-mentioned findings, she was clinically diagnosed as BS type III with the presentation of inactivity, hypokalemia and metabolic alkalosis along with normal renal function.

Administration of intravenous KCl 60 mmol/day followed by oral KCl 1.5 mEq/kg/day

Table 1: Serial chemical data of the patient's blood

Index (Normal range)	Before admission	1st day after admission	8th day after admission	3 months after follow-up	6 months after follow-up	9 months after follow-up
pH(7.34–7.45)	7.45	7.410	7.40	7.367	7.37	7.43
Na ⁺ (135–147 mmol/l)	131	140	137	141	139	144
K ⁺ (3.5–5.3 mmol/l)	2.2	2.6	2.9	3.0	2.7	3.1
Cl ⁻ (95–108 mmol/l)	96	109	107	102	99	95
HCO ₃ ⁻ (20–26 mmol/l)	28	27.2	29	32.6	25	31
Total Ca ²⁺ (1.05–1.35 mmol/l)	1.06	1.06	1.22	1.20	1.12	1.27
Mg ²⁺ (2.0–4.0 mg/dl)	0.42	1.82	2.2	2.01	2.4	2.8
BUN (2.10–7.90 mmol/l)	8	3.52	4.15	6.2	5.9	4.7
Creatinine (22.0–97 mmol/l)	1.6	36.4	51.7	57.2	36.1	82.9

Table 2: The data of Aldosterone, Renin, Angiotensin I and Angiotensin II in the patients in different postures

Parameter	In upright posture		In recumbent posture	
	Patient	Normal range	Patient	Normal range
Aldosterone pg/ml	108.94	59-199	120.36	65-296
Renin ng/ml/h	10.55	1.95-3.99	9.2	0.05-0.79
Angiotensin I ng/ml	22.67		7.36	
Angiotensin II pg/ml	596.75	55.3-115.3	149.37	28.2-52.5

restored and maintained her serum K⁺ concentration at about 3.0 mmol/l. Fluids were also supplemented. Indomethacin (2 mg/kg/day) and spironolactone (2 mg/kg/day) were also regularly given to control the hypokalemia until now. She did not experience any episodes of Indomethacin overdose or received other potential nephrotoxic medications during therapy periods. The most prominent laboratory data was persistent hypokalemia during one year of follow-up till now, especially after the episodes of acute upper respiratory infection and acute gastroenteritis. Her renal function still persisted in good condition.

Molecular analysis: The human-investigation committee of the Zhejiang University School of Medicine approved the study. The parents were informed about the purpose of this study and gave consent. The *polymerase chain reaction (PCR)* for amplifying DNA sequences and direct sequencing of all the exons of *CLCKB* gene was performed using peripheral blood genomic DNA of the patient and her non-consanguineous parent. All the primers were designed according to the sequence of NG_013079.1 (Table 3). Several reported SNPs

and some new nucleotide variations were also found in the *CLCKB* gene of the patient, her mother and father (Table 4). Three of the nucleotide variations are synonymous mutations or silent SNPs, the other nucleotide variations can make the amino acids change, however, these new nucleotide variations were found in the patient and her healthy parents, we concluded that these new nucleotide variations may be SNPs.

Family tree of new mutation or SNPs in exon 20 of *CLCKB* gene was also investigated in this family (Table 5). A novel heterozygous C to G transition at nucleotide 2471 in exon 20 were found in the patient, which has not been detected in her mother or her father (Table 5, Fig. 1). Sequencing for reverse DNA strands confirmed the above result.

Discussion

Bartter's syndrome encompasses a group of closely related inherited tubulopathies

Table 3: The primers for amplifying the *CLCKB* gene in the study

	Forward primer	Reverse primer	Length(bp)	Tm(°C)
Exon1	GGACATTCTAAGTGTTCCGCATAA	CCCAGAAGAAGATCCCACCAG	269	59
Exon2	ACACCCCTCAGTGACGGAAAC	TGCCACCCAGGAGACTTT	446	59
Exon3	CCACTGTCACCTCCCACAAAT	GCCCAGGATCACAGAGCAAG	481	61
Exon4	TGGGTGCCTCCCTGATAC	TCCTGGGCCTTTGTCTGT	265	61
Exon5	ATCTGGCGAGATCGTAATG	GGTAGGGTGGTTGGGATG	276	62
Exon6、7	CAAAAGCCATCTGAGGACG	GAGGGGAGGAGCTTGAGG	424	65
Exon8	TGACTTGATTGGCGGTGCTA	CCCTCCATCCTCCAGAAG	646	59
Exon9、10	TTAGTCTGGGACTTGAGTTTGGG	GGTTGGGAGTACGGTGAGGG	431	59
Exon11	TTGGGATGTGGGAAAGGG	AAAGGAGGCAACAAATAGGG	194	59
Exon12	GGGAAGTGGCAGAGGAGGA	CAGGAAATGTGGGTGGAAGG	393	62
Exon13、14	TGTCCTGTCTCCCTTGT	CCCTGACCCACTCACCT	430	62
Exon15	ACTCCCTCGTGGCTCCTGT	AGCTACGGTGGCGTTTCTTT	516	61
Exon16	TAGGAGTCCCTCATTCCAG	CACGGTTGGTTGCTAAGTC	403	60
Exon17、18	CCCATAGGAACACCAGAACA	GCAGGAAGCAGCATAAGACG	552	60
Exon19	ACCTTCTACCTCCAGTGTTT	TCATCAGTATCCTCTGAATCCC	259	59
Exon20	CCCTCACAACCTCCTTACATC	TGACCACGGGAAACTCACTCT	588	60

Table 4: Genotypes in CLCNKB gene from the patient's family (compared to Genebank accession NG_013079.1)

	Reported SNPs	Other nucleotide variations or new SNPs
Patient	rs2015352 (exon 2), rs5257 (exon 4), rs2014562(exon 5), rs71493533(exon 8), rs5253, rs5254, rs2275166 and rs2275167 (exon 16), rs79198735 (exon 19), rs112496366, rs1057839, rs79768787 and rs1057854 (exon 20).	a homozygous C to T (gcg to gtg) transition at nucleotide 996(exon 9), homozygous T to C (tgt to tgc; synonymous mutation) transition at nucleotide 1012 (exon 10), heterozygous T to C (ggt to ggc; synonymous mutation) transition at nucleotide 1759, heterozygous GC to AT (cgc to cat) transition at nucleotides 1767-8, and homozygous A to G (aag to gag) transition at nucleotide 1868 (exon 16), homozygous C to A (cac to caa) transition at nucleotide 2071, homozygous G to A (gag to aag) transition at nucleotide 2081, a homozygous T to G (cat to cag) transition at nucleotide 2098 and a homozygous G to A (acg to aca; synonymous mutation) transition at nucleotide 2113(exon 19)
Mother	Same as above	Same as above
Father	Same as above	Same as above

characterized by markedly reduced NaCl transport^[4]. The clinical manifestations of the syndrome varied from lack of symptoms to severe growth retardation^[5]. Volume depletion activates the renin-angiotensin-aldosterone system in BS. Renin contributes to renal fibrosis through triggering proliferation of mesangial cell^[6].

Angiotensin II plays a crucial role in renal injury by increasing intraglomerular capillary pressure and promoting fibroproliferative effects. Aldosterone directly accelerates renal damage^[7]. Racial and environmental differences, genetic background, as well as different types of mutation in CLCNKB gene, should be probably involved in the susceptibility to the deterioration of renal function^[8]. Patients with BS are usually associated with high PGE2 production and hypercalciuria^[9]. Chronic non-steroid anti-inflammatory drugs (NSAID) administration in aBS and/or BS usually achieved several benefits such as amelioration or correction of hypokalemia, reduction of hypercalciuria, and preservation of renal function. Cyclo-oxygenase inhibitors such as indomethacin have been recommended as the standard treatment for aBS and/or BS because renal prostaglandin is overproduced. Some patients with BS type III had the trend of patholo-

proteinuria and impaired kidney function after a median of 14 years follow-up^[10]. Till now, renal function and histological data were unaffected in our patients after indomethacin treatment for more than one year. Chronic NSAID administration can be considered in this patient if necessary.

In the kidney approximately 30% of NaCl reabsorption takes place in the thick ascending limb of the loop of Henle and this depends upon a chloride channel complex encoded by CLCNKB and BSND^[11]. To date, more than 30 CLCNKB mutations have been reported in BS^[4,12]. Wright's fixation index (F_{st}) increases as the allele frequency difference between populations increases^[13]. The F_{st} values of rs2003943, rs6604910 and rs1414499 in CLCNKB gene were greater than rs2050522 in the Han Chinese, which exhibited strong linkage disequilibrium^[14]. A total of 13 reported single nucleotide polymorphisms (SNPs) were found in the patient, her father and mother (Table 4), these SNPs were located close to rs2050522, making it a reasonable proxy for surrounding markers, so it is necessary to analyze linkage disequilibrium among these markers in Chinese Han population. A homozygous G470E mutation in the CLCNKB gene was reported in a

Table 5: Genotypes of exon 20 in CLCNKB gene from the patient's pedigree

patient's pedigree	Genotype at 2260 bp of exon 20	Genotype at 2336 bp of exon 20	Genotype at 2363 bp of exon 20	Genotype at 2471 bp of exon 20
Father	G/A	C/C	T/T	C/C
Mother	G/G	C/C	A/T	C/C
Patient	G/G	C/C	A/T	G/C

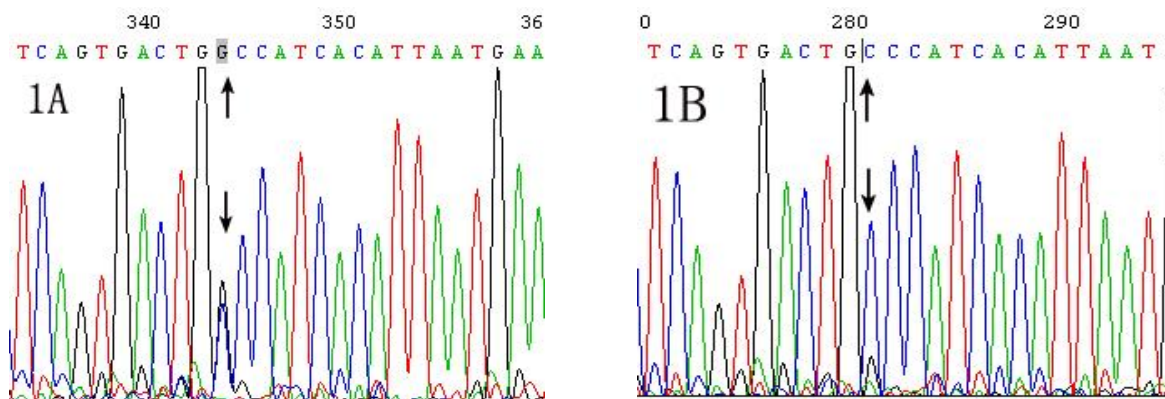


Fig. 1: The new mutation in exon 20 of CLCNKB gene from the patient. Sequencing of the exon 20 in CLCNKB gene was performed in the family. **Fig. 1A:** A heterozygous C to G transition (black arrow, forward primer) at nucleotide 2471 in exon 20 of CLCNKB gene was shown in the patient, which was also confirmed by sequencing for reverse DNA strands. **Fig. 1B:** C/C genotype at nucleotide 2471 in exon 20 of CLCNKB gene was shown in her father (black arrow, forward primer). (Genebank accession NG_013079.1).

Chinese boy^[15], however, G470E mutation was not found in our patient.

Some new nucleotide variations were also identified (Table 4 and 5), among them, three were synonymous mutations or SNPs, the other nucleotide transitions may result in amino acid sequence variations, for example, a homozygous A to G transition at nucleotide 1868 (aag to gag; Lys to Glu). Because these new nucleotide variations were found both in the patient and her healthy parent, we concluded these new nucleotide variations may be new SNPs. The clinical manifestations of BS III were heterogeneous without a genotype-phenotype correlation in a nationwide cohort of 26 Korean children^[16]. The molecular diagnostic steps for patients with BS in our population should be designed taking these peculiar genotype distributions into consideration.

Synonymous SNPs do not produce altered coding sequences, and therefore they are not expected to change the function of the protein in which they occur. However, Kimchi-Sarfaty C reported that a synonymous SNP in the multidrug resistance 1 gene encoding P-glycoprotein (P-gp) affects the timing of cotranslational folding and insertion of P-gp into the membrane, thereby altering the structure of substrate and inhibitor interaction sites^[17]. It demonstrates for the first time that naturally occurring silent SNPs can lead to the synthesis of protein product with the same amino acid sequence but different structural and functional properties. Synonymous mutations have significant consequences for cellular processes^[18]. Thus, silent SNPs in this study

should not be neglected in determining the likelihood of development of BS. Direct sequencing also revealed a novel heterozygous C to G mutation at nucleotide 2471 in exon 20 (the last exon) of CLCNKB gene in the patient, which did not exist in her parent (Table 5, Fig 1).

CLC C-terminal cytoplasmic domains are required for CLC channel function^[19]. The location of a novel compound heterozygous mutation in the exon 20 of CLCNKB gene may result in helix instability of mRNA that leads to a loss of translation of protein. It is also possible that the compound heterozygous mutation disrupts a direct interaction with the membrane domain.

Conclusion

We have identified a novel CLCNKB mutation and some new SNPs in a Chinese girl with BS type III having persistent hypokalemia. SNPs and a novel mutation make the genetic background of the patient more complicated. Further functional study of the mutation and/or SNPs should be taken into account.

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