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CASE REPORT

A New Case Report of a CLCNKB Complex Heterozygous Mutation in Adult-Onset Type III Bartter Syndrome

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SUMMARY

Background: **Type III Bartter syndrome (BS) is an autosomal recessive renal tubular disease caused by the mutation of the chloride voltage-gated channel Kb (CLCNKB) gene. This condition is characterized by renal sodium loss, hypokalemia, metabolic alkaliosis, high renin, and high aldosterone levels.**
Methods: **We report a case of adult type III BS caused by a novel complex heterozygous mutation of the CLCNKB gene. The peripheral blood was extracted for whole genome DNA extraction, and the genome exon region of BS-related genes, was predicted by high-throughput sequencing and protein function prediction software. The selected mutation sites were verified by sequencing with Sanger method.**
Results: **The new complex heterozygous mutations of CLCNKB include heterozygous deletion of exon 2 - 20 of CLCNKB and nonsense mutation of exon 19, c.2010G>A (p.W670X). This complex heterozygous mutation has not been reported in humans.**
Conclusions: **For patients with high clinical suspicion of BS, a clear diagnosis should be made through genetic testing to improve patients' quality of life and provide genetic guidance.**
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KEYWORDS

bartter syndrome, hypokalemia, CLCNKB, complex heterozygous mutation

INTRODUCTION

Bartter syndrome (BS) is a genetic renal tubular disease characterized by impaired electrolyte transmembrane transport in the coarse ascending branch of myelin loop and/or distal convoluted tubules, with an incidence of one in a million [1]. In 1962, Bartter et al. [2] first reported two cases with clinical characteristics of hypokalemia, metabolic alkali poisoning with aldosteronism, and paraglomerular organ hyperplasia that were diagnosed with BS. According to molecular genetics, it is currently classified as I - V type [3]. Considering the large phenotypic variation and different clinical manifestations, clinical diagnosis is difficult. This study involves a case of adult type III BS caused by a novel complex heterozygous mutation of the CLCNKB gene,

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extending the spectrum of CLCNKB variants in BS.

CASE PRESENTATION

Clinical manifestations

The proband, male, 37 years old, was admitted to Deqing People's Hospital on May 9, 2022, because of "low blood potassium for one day after physical examination". The blood potassium concentration during physical examination was 2.49 mmol/L (normal range: 3.5 - 5.3 mmol/L) without obvious discomfort. The patient has no history of hypertension. The patient also has no history of special drug use, has deceased parents, has no siblings, is unmarried, and is childless. Based on physical examination, the blood pressure was 122/80 mmHg, and the body mass index was 19.9 kg/m^2. Based on limb strength level V, the muscle tone is normal. For the auxiliary test results, the blood potassium was 2.71 mmol/L, blood sodium was 139 mmol/L (normal range: 137 - 147 mmol/L), blood calcium was 2.31 mmol/L (normal range: 2.11 - 2.52 mmol/L), blood magnesium was 1.78 mmol/L (normal range: 1.24 - 1.79 mmol/L), blood chlorine was 100 mmol/L (normal range: 99 - 110 mmol/L), simultaneous 24-hour potassium was 67.0 mmol/L (normal range: 25 - 100 mmol/L), 24-hour sodium 118 mmol/L (normal range: 110 - 220 mmol/L), and 24-hour urinary calcium was 3.7 mmol/L (normal range: 2.7 - 7.5 mmol/L). Based on blood gas analysis, the pH was 7.454, actual bicarbonate was 29.2 mmol/L, standard bicarbonate was 27.7 mmol/L, and alkali residual was 5.0 mmol/L. We also observed 24-hour urine-free cortisol, blood cortisol rhythm, blood liver and kidney function, myocardial enzyme profile, and no significant abnormalities in thyroid function. Two evaluations of renin activity-aldosterone in the erect position: The first examination revealed that renin activity in the erect blood was 14.58 μg/L/hour (reference range: 1.31 - 3.95), and aldosterone in the erect position was 313.77 ng/L (reference range: 50 - 313); the second evaluation indicated that the renin activity, in the erect position, was 9.16 μg/L/hour, whereas the aldosterone, in the erect position, was 303.51 ng/L. Adrenal color ultrasound and adrenal enhanced CT showed no abnormality. Blood pressure monitoring during hospitalization indicated fluctuations within the normal range (109 - 122/75 - 80 mmHg). Considering that the patient suffers from severe hypokalemia combined with metabolic alkalosis, renal loss of potassium, elevated serum renin activity and aldosterone, and no hypomagnesium and hypourinary calcium, the diagnosis of BS was considered. Gene sequencing was conducted after communication with the patient to confirm the diagnosis. At the same time, the patient was prescribed 25 mg indomethacin tablets thrice a day and 1.0 g of potassium chloride sustained-release tablets twice a day orally. The patient was followed up for 3 months for blood potassium monitoring (Table 1).

Genetic test results

With informed consent, blood from the proband was extracted for peripheral blood whole genome DNA extraction. The genome exon region of BS-related genes (*SLC12A1*, *KCNJ1*, *CLCNKB*, *BSND*, and *CASSLC1 2A3*) was predicted by high-throughput sequencing and protein function prediction software. The selected mutation sites were verified by sequencing with the Sanger method (detected by Beijing Maikino Medical Laboratory). The results show that: 1) The progenitor had a heterozygous deletion of CLCNKB gene (chr1:16370 687-16383711) exon No. 2 - 20 (Figure 1A), and this mutation was a zero-effect mutation. According to the guidelines of the American Society for Medical Genetics and Genomics (ACMG) [4], this mutation was a pathogenic mutation. Cases of incognito inheritance of this mutation have been reported, the frequency of this mutation in the normal population database has low frequency variation, and the bioinformatics protein function prediction software REVEL was unknown. 2) The progenitor had a nonsense mutation c.2010G>A (p.W670X) in the CLCNKB gene (chr1:16382997) exon 19 (Figure 1B), resulted in the encoding of nucleotide 2010 changing from guanine G to adenine A, which was a zero-effect mutation. According to the ACMG guidelines, the mutation was suspected to be pathogenic, and no correlation of this locus was reported in the literature database. The frequency in the normal population database was low-frequency variation, and the prediction of bioinformatics protein function prediction software REVEL was unknown. Therefore, deletion 2-20+ c.2010G>A (p.W670X) is a new complex heterozygous mutation of CLCNKB gene. Blood was not obtained for sequencing due to deceased parents.

DISCUSSION

BS is an autosomal recessive inherited renal tubular disease caused by mutations in genes encoding ion channels or transporters. This condition can be classified into types I, II, III, IVa, IVb, and V according to different molecular genetic causes [5]. Type III BS, also known as meridian syndrome (cBS), is caused by mutations in the CLCNKB gene. The CLCNKB gene is located on the lp36 chromosome and encodes voltage-gated chloride ion channels (CLC-Kb) [6]. CLC-Kb is mainly expressed in the basal membrane side of epithelial cells in the ascending branch of renal pulp loop, distal curved tubules, connecting tubules, and collecting tubules and forms an open chloride channel with β-subunit barttin; it is responsible for chloride ion and other electrolyte transport [7]. CLCNKB gene mutation results in decreased sodium chloride reabsorption and insufficient extracellular fluid volume in renal tubules after the inactivation of CLC-Kb, resulting in increased activity of the renin-angiotensin-aldosterone system (RAAS), leading to hypopotassium and hypochloride alkalosis [1]. cBS usually occurs in infants and early children, and is

Table 1. Electrolyte changes before and after treatment.

Temporal	Potassium (mmol/L)	Sodium (mmol/L)	Chloride (mmol/L)	Calcium (mmol/L)	Treatment plan
2022/5/8	2.49	139	100	2.31	Potassium chloride sustained release Tablets 1.0, three times/day
2022/5/9	2.34	140	97	2.32	Potassium chloride sustained release Tablets 1.0, three times/day
2022/5/24	3.43	140	97	2.39	Potassium chloride sustained-release Tablets 1.0, three times/day + indomethacin tablets 25 mg, three times/day
2022/6/21	3.26	140	99	2.37	Potassium chloride sustained-release Tablets 1.0, two times/day + indomethacin tablets 25 mg, three times/day
2022/7/5	3.28	140	101	2.26	Potassium chloride sustained-release Tablets 1.0, two times/day + indomethacin tablets 25 mg, three times/day
2022/8/2	3.28	142	102	2.41	Potassium chloride sustained-release Tablets 1.0, two times/day + indomethacin tablets 25 mg, three times/day

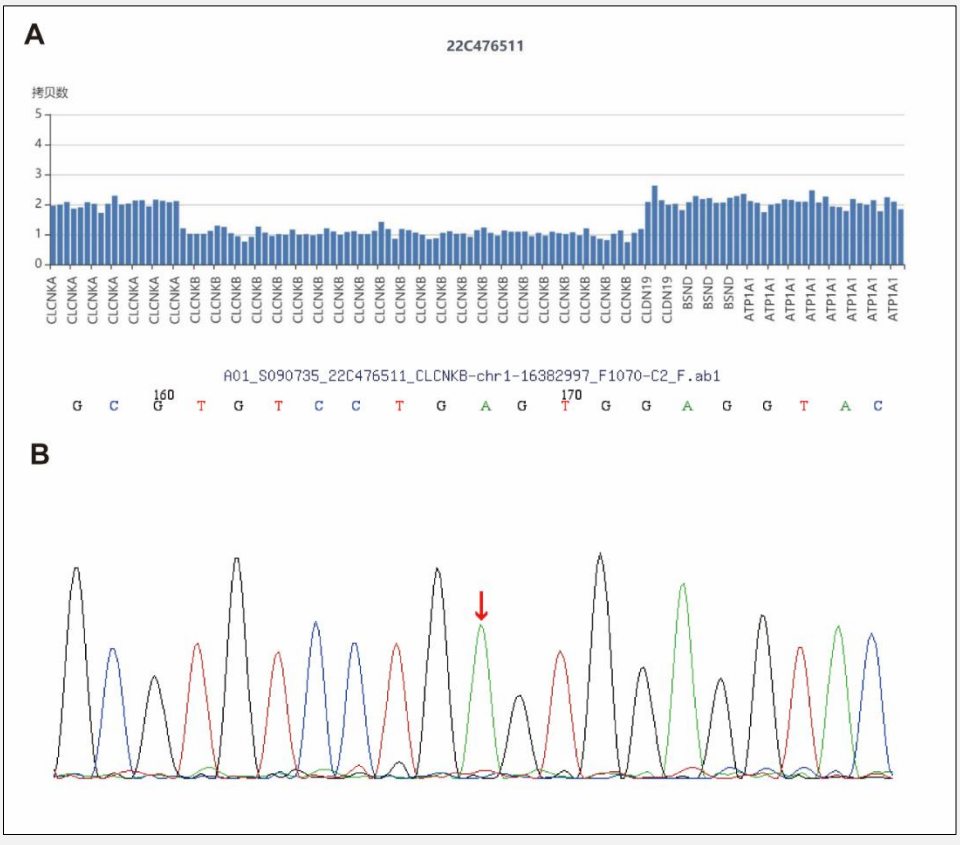

Figure 1. Genetic test results.

(A) Sequencing of the deletion of exon 2 - 20 of the progenitor CLCNKB gene, and (B) nonsense mutation of prover CLCNKB gene exon 19 (c.2010G>A; p.W670X).

rare in adults. It can cause polydipsia, polyuria, growth retardation, and muscle weakness and is usually not accompanied by renal calcium deposition [5]. However, BS is heterogeneous in terms of clinical phenotype and genetic phenotype, and its clinical phenotype varies greatly. CLCNKB mutation may also lead to the overlap of clinical phenotype with Gitelman syndrome, and the clinical phenotype may be related to the mutation type [8,9]. Genetic testing is the gold standard for diagnosis. Nearly 100 CLCNKB gene mutations have been reported according to the human gene mutation library. Currently, 13 types of CLCNKB gene mutations have been reported in type III BS, including intron mutation, missense mutation, nonsense mutation, deletion mutation, and repeated mutation [10,11]. These mutations lead to impaired or reduced expression of ClC-Kb chloride channels on cell membranes and reduced function of chloride channels [12].

The patient was diagnosed as an adult with mild clinical manifestations. Laboratory examination suggested hypokalemia, renal dyskalemia, metabolic alkalosis, hyperreninemia, and hyperaldosteronemia. BS was first considered in diagnosis. Through genetic testing, this condition can be identified as the type III BS caused by complex heterozygous mutation of CLCNKB gene. The deletion 2 - 20+ c.2010G>A (p.W670X) complex heterozygous mutation of CLCNKB gene was found for the first time in the population. Deletion 2 - 20 is the deletion of large fragments of genes, which is a pathogenic variation that can lead to the loss of gene function [4]. c.2010G>A (p.W670X) is a semi-zygotic mutation that results in changes in amino acids and possibly loss of gene function. Combined with the light clinical phenotype of the patients, the complex heterozygous mutation of the CLCNKB gene may have relatively little effect on ClC-Kb chloride channel protein, and the specific significance of the mutation needs to be clarified by perfect functional tests. We report a case of adult type III BS caused by a new complex heterozygous mutation of CLCNKB gene, which extends the spectrum of CLCNKB variants in BS. Unfortunately, the patient's parents were deceased. Consequently, gene verification could not be performed.

At present, no radical treatment, which mainly focuses on symptom control, is available for BS. Classical drug treatment includes supplementary potassium chloride, prostaglandin inhibitors, and aldosterone antagonists [13]. Foods that are high in potassium are recommended in the diet. After treatment, hypokalemia still persists, but the condition is better than that before treatment, and the reasonable target level of blood potassium is approximately 3.0 mmol/L [14,15]. After treatment with potassium chloride and indomethacin, blood potassium fluctuated between 3.01 mmol/L and 3.69 mmol/L.

In conclusion, for patients with high clinical suspicion of BS, a clear diagnosis should be made through genetic testing. Correct diagnosis and treatment can improve patients' quality of life and provide genetic guidance.

Informed Consent:
Written informed consent was obtained from the individual for the publication of any potentially identifiable images or data included in this article.

Key Clinical Message:
To raise awareness of this rare and life-threatening clinical symptom, we report a 37-year-old male onset with recurrent hypokalemia, clinically diagnosed Type III Bartter syndrome (BS), and genetically confirmed new complex heterozygous mutations of CLCNKB. This complex heterozygous mutation has not been reported in humans.

Declaration of Interest:
There is no conflict of interest to disclose.

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