

A Novel Homozygous *CLCNKB* Mutation of Classic Bartter Syndrome Presenting with Renal Cysts in 6-year-Old Identical Twin Boys : A Case Report

Min Hwa Son, M.D.
Hyung Eun Yim, M.D., Ph.D.
Kee Hwan Yoo, M.D., Ph.D.

Department of Pediatrics, Korea
University College of Medicine, Seoul,
Korea

Corresponding author: Hyung Eun Yim,
M.D., Ph.D.

Department of Pediatrics, Korea University
Ansan Hospital, Korea University College
of Medicine, 123 Jeokgeum-ro, Danwon-
gu, Ansan 15355, Republic of Korea

Tel: +82-31-412-5096

Fax: +82-31-405-8591

E-mail: he-yim@hanmail.net

Received: 26 January 2021

Revised: 19 February 2021

Accepted: 8 March 2021

Bartter syndrome is an autosomal recessive hypokalemic salt-losing tubulopathy, and classic Bartter syndrome is associated with mutations in the *CLCNKB* gene. While chronic hypokalemia is known to induce renal cyst formation in different renal diseases, renal cyst formation in Bartter syndrome is rarely reported. Russian six-year-old identical male twins were referred to our hospital for the evaluation of renal cysts, which were incidentally detected on abdominal sonography due to diarrhea. Both twins had shown symptoms of polydipsia, polyuria, and nocturia since they were one year olds. Vital signs including blood pressure were normal in both twins. Renal sonography revealed nephromegaly, increased echogenicity of renal cortex, and various sized multiple cysts in both kidneys for both twins. Laboratory findings included hyponatremia, hypokalemia, hypochloremia, and metabolic alkalosis. Bartter syndrome with renal cysts were suspected. Genetic analysis for both twins confirmed a homozygous c.1614delC deletion on exon 15 of the *CLCNKB* gene, which was confirmed as a previously unreported variant to the best of our knowledge. They were managed with potassium chloride, nonsteroidal anti-inflammatory drugs, and angiotensin-converting-enzyme inhibitors. Metabolic alkalosis, hypokalemia, hypochloremia, and polyuria partially improved during the short course of treatment. This is the first report of a homozygous mutation in the *CLCNKB* gene in an identical twin, presenting with renal cysts.

Key words: Bartter syndrome, Type 3, Chloride channels, Cysts, Hypokalemia, Twins, Monozygotic, Case report

Introduction

Bartter syndrome is a rare hereditary hypokalemic tubulopathy. It presents with two broad phenotypes, one with antenatal Bartter syndrome, and the other with classic Bartter syndrome, typically associated with mutations in the *CLCNKB* gene¹⁾. While all forms of Bartter syndrome are characterized by hypokalemic, hypochloremic, and hyperreninemic metabolic alkalosis with normal blood pressure, the clinical presentation of classic Bartter syndrome is very heterogeneous, ranging from very mild to severe²⁾. Here, we report a Russian 6-year-old identical twin who presented with severe hypokalemia and multiple renal cysts, and they were found to have Bartter syndrome with a novel homozygous c.1614delC deletion on exon 15 of the *CLCNKB* gene.

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Case report

A six-year-old male identical twin from Russia visited our hospital for the evaluation of renal cysts. Kidney cysts were incidentally found throughout the evaluation of acute diarrhea in Russia 6 months ago. The twin boys had no specific pre-natal and perinatal histories. At 18 months of ages, they were evaluated for short stature in Russia, which showed no specific findings. Since two years of age, both boys developed polydipsia, polyuria, and nocturia. They were not on any medications, and their family history was unremarkable. The first twin's height was in the 7th percentile and body weight was in 16th percentile. His blood pressure levels were normal. There were no specific findings on physical examination. The laboratory findings for the first twin showed metabolic alkalosis (pH 7.545 and HCO_3^- 31.6 mmol/L), hyponatremia (131 mmol/L), severe hypokalemia (2.3 mmol/L), hypochloremia (81 mmol/L), and normal serum magnesium level (2.1 mg/dL). Transtubular potassium gradient (TTKG, 12.2) and fractional excretion of chloride (2.56%) were elevated. Urinary calcium excretion was normal. Renin was elevated (30.48 ng/mL/hr, normal 0.8–2.0 ng/mL/hr), but aldosterone was normal (26.4 ng/dL, normal ≤ 40 ng/dL). Urine β_2 -microglobulin was increased (2.57 $\mu\text{g/mL}$, normal 0–0.3 $\mu\text{g/mL}$). The

second twin also had hyponatremia (133 mmol/L), hypokalemia (2.5 mmol/L), and hypochloremia (83 mmol/L). The total CO_2 level was 29.8 mmol/L. The levels of magnesium were within the normal range. The renal potassium and chloride wasting was also found with TTKG of 14.4 and urine chloride of 43 mmol/L. Renin (30.48 ng/mL/hr) level was elevated and aldosterone (26.4 ng/dL) was normal. Electrocardiogram showed mild corrected QT prolongation, however, echocardiographic findings showed normal findings. Renal sonography of both boys showed nephromegaly with increased cortical echogenicity and multiple cysts of varying sizes in both kidneys (Fig. 1A-D). Although cystic kidney diseases such as autosomal recessive polycystic kidney disease (ARPKD) were initially suspected on renal sonography, their laboratory and clinical findings were consistent with Bartter syndrome. There were no specific findings compatible with ARPKD. Therefore, they were diagnosed with Bartter syndrome with renal cysts. Due to financial problem, only the first twin was admitted to the hospital for the treatment of severe hypokalemia. After admission, serum potassium and chloride levels did not increase effectively, despite vigorous potassium (5.0 mEq/kg/d) and chloride (7.09 mEq/kg/d) supplementation. Renal potassium wasting and polyuria persisted (urine volume 3.11 L/m²/day). Non-steroidal anti-inflammatory

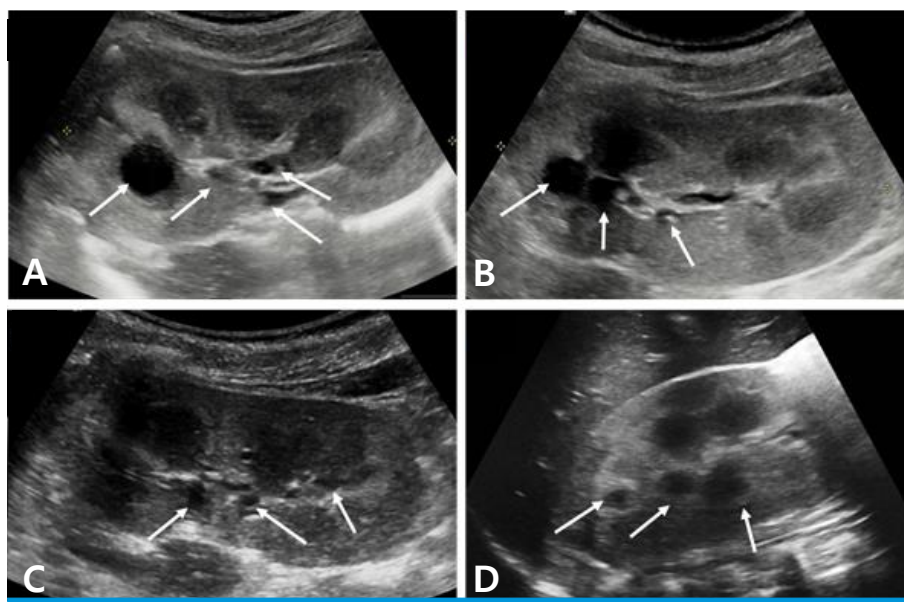


Fig. 1. Renal ultrasonography The ultrasound pictures of both boys show large, echogenic kidneys with multiple cysts (white arrows) of various sizes measuring 0.53–1.93 cm. A: Left kidney of first twin (length 9.32 cm) B: Right kidney of first twin (length 9.19 cm) C: Left kidney of second twin (length 9.37 cm) D: Right kidney of second twin (length 9.0 cm).

drug (ibuprofen) and angiotensin-converting enzyme inhibitor (lenipril, 0.13 mg/kg/day) were added from the third day of admission. The hourly urine output was reduced from 9.0 mL/kg/hr to 3.09 mL/kg/hr following the administration of ibuprofen (Fig. 2A). The boy was discharged with mildly improved metabolic alkalosis, hypokalemia and hypochloremia on the fourth hospital day (Fig. 2B). Potassium chloride (K-contin, 2.56 mEq/kg/day), lenipril (0.13 mg/kg/day), and ibuprofen were prescribed. The second twin who was treated through an outpatient clinic was also prescribed potassium chloride (K-contin, 3.08 mEq/kg/day), lenipril (0.1 mg/kg/day), and ibuprofen. They returned to Russia with a 30-day medication and a referral form. The genetic analysis for both boys revealed a homozygous c.1614delC deletion on exon 15 of the *CLCNKB* gene, which is a previously unreported variant to the best of our knowledge (Fig. 3A and B). *CLCNKB*

gene encodes the kidney-specific chloride channel CLC-Kb.

Discussion

This is a case report of classic Bartter syndrome in an identical twin who presented with renal cysts, polyuria, polydipsia, and hypokalemic, hypochloremic and hyperreninemic metabolic alkalosis with normal blood pressure. While ARPKD was suspected initially, their clinical and laboratory findings excluded ARPKD but suggested Bartter syndrome. The genetic analysis for both boys confirmed a novel homozygous mutation of the *CLCNKB* gene.

Bartter syndrome is a rare AR salt-losing tubulopathy, identified by Frederic Bartter in 1962³. It is clinically categorized into antenatal or classic Bartter syndrome, and five genetic subtypes with mutations in *SLC12A1*, *KCNJ1*,

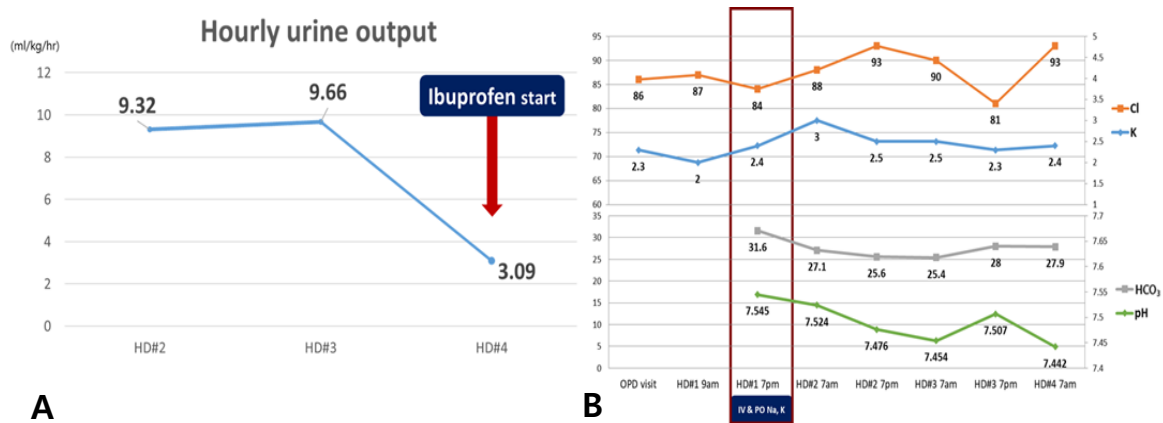


Fig. 2. A, Change HUU after ibuprofen; B, Improvement for metabolic alkalosis, hypokalemia and hypochloremia.

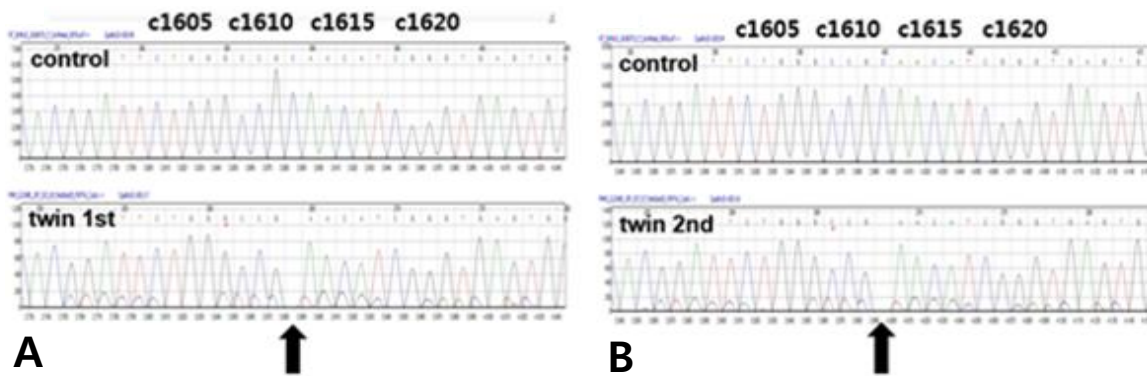


Fig. 3. Mutation analysis. Mutation analysis of both twin showed a homozygous c.1614delC deletion on exon 15 of the *CLCNKB* gene (black arrows). A, Analysis of mutation in the *CLCNKB* gene of first twin; B, Analysis of mutation in the *CLCNKB* gene of second twin.

CLCNKB, *BSND*, and *CASR* have been identified to date⁴. These are all autosomal recessive types, but recently X-linked inheritance-type Bartter syndrome was revealed, which is mutations in the gene encoding the protein melanoma associated antigen D2 (*MAGE-D2*)⁵. Classic Bartter syndrome is caused by the mutation in *CLCNKB* in the chloride channel family of genes. Its features for older children include failure to thrive, dizziness, polyuria, polydipsia, non-specific fatigue and muscle weakness¹. To the best of our knowledge, there have been no cases of classic Bartter syndrome presenting with multiple renal cysts in identical twins, as in our case. The twins showed previously unreported mutation on exon 15 of the *CLCNKB* gene.

Notably, renal cysts are frequent findings in patients with chronic hypokalemia owing to hyperaldosteronism or other renal potassium-wasting disorders^{6,7}. In a study analyzing the 55 patients with hyperaldosteronism⁶, 44.4% had renal cysts. Lower serum potassium levels and higher plasma renin activity independently predicted the presence of kidney cysts. In addition, the number and size of renal cysts were reduced strikingly after adrenal adenomas were removed, which was consistent with the normalization of the serum potassium levels. Meanwhile, renal cyst formation is rarely reported in Bartter syndrome, but, in 2005, a case of bilateral renal cysts and nephrocalcinosis was described in a 6-year-old girl with classic Bartter syndrome⁸. She had a heterozygous nonsense mutation (W610X) on exon 16 of the *CLCNKB* gene. While nephrocalcinosis is almost invariable in antenatal Bartter syndrome, the authors suggested that nephrocalcinosis may develop in patients with classic Bartter syndrome and that both hypokalemia and nephrocalcinosis might contribute to the development of renal cysts⁸. Our twin case did not show any evidence of nephrocalcinosis, but they had various sizes of multiple renal cysts and mutation of the *CLCNKB* gene. Persistent severe hypokalemia seemed to be associated with the presence of renal cysts in this clinical setting.

Renal cysts may develop as a complication of deregulated solute transport and increased renal cell proliferation under the condition of chronic hypokalemia. Decreased extracellular potassium concentration enhanced renal cell proliferation in vitro and collecting ductal hyperplasia was observed in potassium-deficient rats^{9,10}. Furthermore, hypokalemia stimulates glutamine uptake and ammoniogenic

enzyme expression in the proximal tubule¹¹. Increased renal ammonia production can stimulate DNA, RNA and protein synthesis, facilitate renal cell growth, and increase interstitial injury during which renal cyst formation may increase¹¹. In both patients, the renal sonography showed nephromegaly, increased parenchymal echogenicity, and multiple various sized cysts. Medical history, clinical presentations, and laboratory findings were sufficient for ruling out any possibility of PCKD. The urinary β_2 -microglobulin level was also elevated, suggesting tubular injury. These findings indicate that persistent severe hypokalemia may play a role in renal tubular cell hypertrophy, tubulointerstitial injury, and cyst formation. Another point to consider is that the renin angiotensin aldosterone system (RAAS) activation can promote the growth of renal cysts through several signaling cascades. Angiotensin II is known to promote vascular hyperplasia, cyst expansion, and intrarenal ischemia, leading to the disruption of the renal architecture¹². Lastly, the mutation of the *CLCNKB* gene in our cases could fundamentally contribute to the renal cystic growth. Chloride channels plays an essential role for cell volume regulation and transepithelial transport, and the novel homozygous deletion on exon 15 of the *CLCNKB* gene in our identical twin cases would cause more severe loss of function than seen with other known mutations. The wide spectrum of functional severity of *CLCNKB* mutations correlated with the phenotypic variability in patients with classic Bartter syndrome¹³. Since functional analyses were performed in only 20 of the *CLCNKB* mutations², further investigation for the impact of *CLCNKB* mutations on the chloride channel activity is necessary.

In patients with Bartter syndrome, comprehensive therapy such as potassium supplementation, prostaglandin inhibitors, RAAS blockers and potassium-sparing diuretic is required⁴. Our patients were treated with potassium chloride, lenipril, and ibuprofen. Metabolic alkalosis, hypokalemia, hypochloremia, and polyuria partially improved during the short course of treatment. Because this family did not revisit our clinic after leaving our country, we could not perform any genetic investigation for the parents. Therefore, we do not clearly confirm whether the mutation was de novo or inherited from their parents. Although Bartter syndrome rarely progresses to end-stage renal dis-

ease, chronic kidney disease with severe proteinuria developed in a substantial number of patients during follow-up^{14,15}). To manage Bartter syndrome in a timely manner, we should carefully take medical history, promptly make a diagnosis, appropriately treat electrolyte imbalance, and finally improve their quality of life.

Conclusion

This is the first report of a novel homozygous mutation in the *CLCNKB* gene in an identical twin, presenting with multiple renal cysts. Although the mechanisms underlying renal cyst formation remain unclear, mutations in the *CLCNKB* gene may reveal variable Bartter phenotypes. Functional study of the mutations and long-term follow-up are necessary to provide targets for individual treatment in patients with Bartter syndrome.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

Patient consent

This study was approved by the institutional review board (IRB), and the consent was waived due to the nature of the retrospective study (IRB number 2019AS0156).

Acknowledgments

We would like to give special thanks to Professor Myung-Hyun Nam (Department of Laboratory Medicine, College of Medicine, Korea University) for carrying out the genetic analysis.

References

- Walsh PR, Tse Y, Ashton E, Iancu D, Jenkins L, Bienias M, et al. Clinical and diagnostic features of Bartter and Gitelman syndromes. *Clinical Kidney Journal* 2018;11:302-9.
- Andrini O, Keck M, Briones R, Lourdel S, Vargas-Poussou R, Teulon J. ClC-K chloride channels: emerging pathophysiology of Bartter syndrome type 3. *Am J Physiol Renal Physiol* 2015;308:F1324-34.
- Bartter FC, Pronove P, Gill Jr JR, MacCardle RC. Hyperplasia of the juxtaglomerular complex with hyperaldosteronism and hypokalemic alkalosis: a new syndrome. *Am J Med* 1962;33:811-28.
- Yang X, Zhang G, Wang M, Yang H, Li Q. Bartter Syndrome type 3: Phenotype-genotype correlation and favorable response to ibuprofen. *Front Pediatr* 2018;6:153.
- Besouw MT, Kleta R, Bockenbauer D. Bartter and Gitelman syndromes: Questions of class. *Pediatric Nephrology* 2019:1-10.
- Torres VE, Young Jr WF, Offord KP, Hattery RR. Association of hypokalemia, aldosteronism, and renal cysts. *N Engl J Med* 1990;322:345-51.
- Gamakaranage CS, Rodrigo C, Jayasinghe S, Rajapakse S. Hypokalemic paralysis associated with cystic disease of the kidney: case report. *BMC Nephrology* 2011;12:1-4.
- Watanabe T, Tajima T. Renal cysts and nephrocalcinosis in a patient with Bartter syndrome type III. *Pediatric Nephrology* 2005;20:676-8.
- Walsh-Reitz MM, Toback FG. Kidney epithelial cell growth is stimulated by lowering extracellular potassium concentration. *Am J Physiol* 1983;244:C429-32.
- Elger M, Bankir L, Kriz W. Morphometric analysis of kidney hypertrophy in rats after chronic potassium depletion. *Am J Physiol* 1992;262:F656-67.
- Han KH. Mechanisms of the effects of acidosis and hypokalemia on renal ammonia metabolism. *Electrolyte Blood Press* 2011;9:45-9.
- Hian CK, Lee CL, Thomas W. Renin-angiotensin-aldosterone system antagonism and polycystic kidney disease progression. *Nephron* 2016;134:59-63.
- Cheng CJ, Lo YF, Chen JC, Huang CL, Lin SH. Functional severity of *CLCNKB* mutations correlates with phenotypes in patients with classic Bartter's syndrome. *J Physiol* 2017;595:5573-86.
- Zhu B, Jiang H, Cao M, Zhao X, Jiang H. A novel *CLCNKB* mutation in a Chinese girl with classic Bartter syndrome: a case report. *BMC Medical Genetics* 2019;20:1-6.
- Bettinelli A, Borsa N, Bellantuono R, Syrèn M-L, Calabrese R, Edefonti A, et al. Patients with biallelic mutations in the chloride channel gene *CLCNKB*: long-term management and outcome. *Am J Kidney Dis* 2007;49:91-8.