Journal of Advances in Medical and Biomedical Research | ISSN:2676-6264

Identification of a Novel *CLCNKB* Mutation in an Iranian Family with Bartter Syndrome Type 3

Javad Mohammadi-asl[1]*, Heshmatolah Shahbazian[2], Farzad Jasemi Zergani[3], Alireza Kheradmand[4]

1. *NoorGene Genetic Lab,Ahvaz, Iran*
2. *Dept. of the Pediatrics, Imam Khomeini Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
3. *Dept. of Internal Medicine, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
4. *Dept. of Urology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

Article Info

10.30699/jambs.30.139.185

Received: 2020/08/26;
Accepted: 2021/03/03;
Published Online: 31 Jan 2022;

Use your device to scan and read the article online

Corresponding Information:

Javad Mohammadi-Asl,
NoorGene Genetic Lab,Ahvaz, Iran
E-Mail: :mohammadi-asl@ajums.ac.ir

ABSTRACT

Bartter syndrome (BS) is a group of uncommon genetic disorders of reabsorption of salt in the cortical thick ascending limb (TAL) of the Henle's loop, typically distinguished by metabolic alkalosis, salt loss, hypokalemia, hyperreninemic hyperaldosteronism and normal blood pressure. Bartter syndrome type 3, recognized as a classic BS (CBS), occurs because of mutations in *CLCNKB* gene.

We enrolled one consanguineous Iranian family with one patient in our study. Targeted genomic capture and massively parallel sequencing (MPS) of all recognized genes responsible for BS subtypes 1–5 were carried out to recognize the genetic reasons of BS.

Here, we report the recognition of a novel homozygous frameshift mutation in the *CLCNKB* gene in an Iranian pedigree. The subjects were homozygous for a frameshift mutation (p.Gly662GlyfsX12) within *CLCNKB* gene that encodes the basolateral chloride voltage-gated channel Kb.

The identification of other causative mutations in *CLCNKB* gene additionally supports the important function of this gene in causing BS. To the best of our knowledge, this is a novel *CLCNKB* gene mutation in BS children. The accurate function of the *CLCNKB* Gly662GlyfsX12 mutation in the CBS pathogenesis is still unknown.

Keywords: Bartter syndrome; mutation;*CLCNKB*; Whole exome sequencing

Introduction

Bartter syndrome is a rare autosomal disorder characterized by deficiencies in distal tubular NaCl reabsorption in the TAL of the Henle's loop (TALH) causing excessive urinary potassium, chloride, and sodium excretion. BS cases usually show hypokalemia, hypochloremia, metabolic alkalosis,hyperreninemia, hyperaldosteronemia and normal blood pressure **(1)**.

BS is clinically categorized into three different variants as follows: 1) Antenatal Bartter syndrome (ABS), also called as hyperprostaglandin E syndrome , is generally the most severe type of BS.It is related to maternal polyhydramnios, premature birth, hypokalemic metabolic alkalosis,systemic symptoms,for example, fever, diarrhea, and failure to thrive,and early onset of nephrocalcinosis. 2) Gitelman Syndrome (GS) which is associated with a milder clinical appearance after the age of 6 yr **(1)**. Patients show with transient muscle weakness, tetanic episodes, abdominal discomfort, symptoms of neuromuscular excitability, or unexplained hypokalemia. On the contrary to the other forms, hypocalciuria and hypomagnesemia are typical **(2)**. 3) Classic Bartter Syndrome which is associated with a noticeable hypokalemic salt-losing resulting in polyuria, polydipsia, muscle weakness and volume contraction, and growth retardation during infancy (birth to 2 years old) or early childhood (3 to 8 years old) **(1, 2)**.

BS is divided into 5 molecular subtypes depending on the mutated gene: Type I occurs because of mutations in *SLC12A1* gene that ecncods a kidney-specific Na-K-Cl(sodium-potassium-chloride) cotransporter NKCC2

(OMIM #601678); Type II occurs because of mutation in *KCNJ1* gene,which encods the apical inwardly rectifying potassium channel ROMK (OMIM #241200); Type III is associated with a mutation in the *CLCNKB* (chloride channel Kb) gene, which encods the basolateral chloride channel ClC-Kb (OMIM #607364); type IVa is related to mutations of the *BSND* gene, which encode the β-subunit for ClC-Ka and ClC-Kb chloride channels (OMIM #602522),often associated with sensorineural hearing loss; type IVb occurs because of a mutation in *CLCNKB* and *CLCNKA* genes (OMIM #613090); Finally, type V is related to a mutation in *CaSR* gene, which encods the basolateral calcium (Ca^{2+}) sensing receptor (OMIM#601199) **(3)**.

In this study, we report an Iranian pedigree with persistent hypokalemia caused by simultaneous homozygous frameshift mutation in *CLCNKB*.

Case Report

A 13- year-old Iranian female with BS was enrolled in the study. The index patient (IV-1) was the first child of a couple with consanguineous marriage (first cousin) originated from southwest Iran (Fig. 1A).The patient was regarded as having BS when she presented with dehydration with hypokalemia,alkalosis,renal salt losing without antenatal symptoms, no significant disturbance of serum calcium (Ca^{2+}) or magnesium (Mg^{2+}) and growth retardation.The patient revealed no progressive muscle weakness of the limbs and was of rather short stature. The birth history of the patient showed full-term normal spontaneous vaginal delivery without antenatal polyhydramnios. There was no family history of BS in the pedigree. Written informed consent form (WICF) was obtained from all participants in the genetic analysis of the correlated genes and the publication of this report based on the guidelines of the Ethics Committee of Iran's Ministry of Health and Medical Education.

Blood samples were collected from the members of the family and genomic DNA (gDNA)was obtained from blood leukocytes by using salting out methods and then was sent for whole-exome sequencing (WES) to investigate the possible cause of BS including analysis of the genes involved in pathogenesis of BS.The effect of candidate variant in protein structure was predicted by using standard global databases and bioinformatics tools like Mutation Taster, SIFT and PolyPhen to estimate the pathogenicity risk of the variant. Also, multiple sequence alignment of human CLCNKB protein by https://www.-uniprot.org/_online tools was used to predict the conservation of the variant among diverse kinds of species. To confirm the novel variant recognized by NGS, direct Sanger sequencing was performed on the patient (IV-1). The specific PCR primers of exon 19 of *CLCNKB* gene previously reported,**(4)** were utilized for the amplification of a site in the DNA of target gene according to the reference genomic sequences in Gene Bank at NCBI (NM_000085.4) and then direct sequencing of PCR products (ABI 3100; Applied Biosystems) was done.

The DNA sequence analysis of the genes involved in pathogenesis of BS in the family by using Whole Exome Sequencing (WES) based on Next Generation Sequencing (NGS) showed one novel mutation in *CLCNKB* gene cosegregating in the affected member of the family. The mutation was a novel homozygous frameshift mutation, c.1984insG, in exon 19 of *CLCNKB* gene (Fig. 1B) which resulted in a frameshift and the substitution of a glycine to glycine substitution with different codonat codon 662 leading to a truncated protein of CLCNKB m-RNA.

DNA sequence analysis of the other genes failed to detect any disease causing sequence variants in the family. Then, the detected novel mutation was confirmed by using Sanger sequencing. The novel mutation alters evolutionary highly conserved amino acids (Fig. 1C). Also, we confirmed that the her unaffected parents and sister (IV-2) had the *CLCNKB*c.1984insG frameshift mutation in heterozygous state.

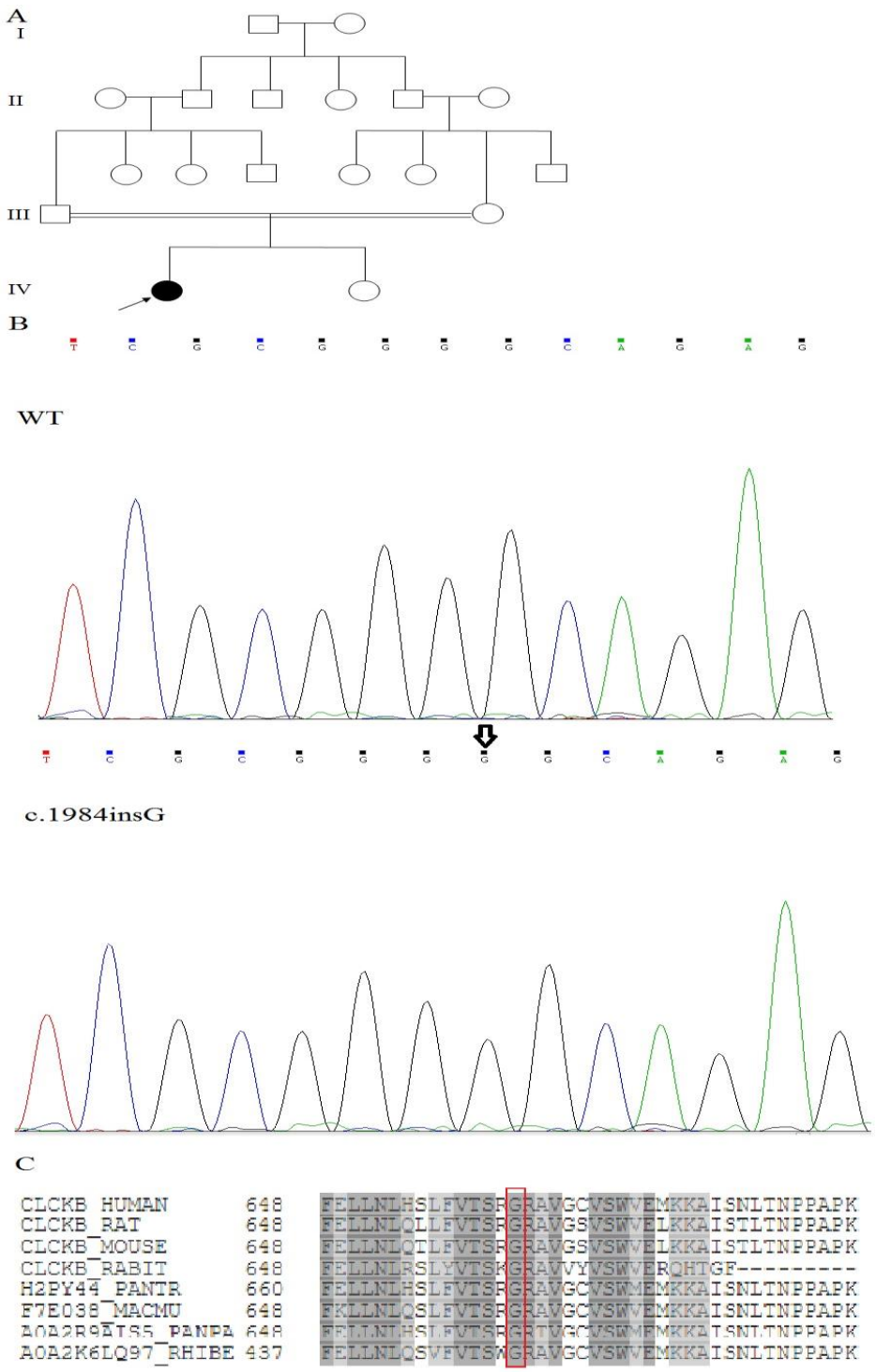

Figure 1. (A The pedigree of family for the c.1984insG mutation. The black symbol indicates affected individual. B). Sanger sequencing show the *CLCNKB* c.1984insG mutation in the proband. Black arrows indicate the mutation.C) Multiple alignments of the *CLCNKB* in different species. The amino acid sequence of human *CLCNKB* is aligned with sequences of other species. The =arrow marks the p.G662Gfs*12 mutation.

Discussion

Type 3 BS is linked to the mutation in the chloride voltage-gated channel Kb gene that encodes a chloride channel protein, a member of the CLC family, called ClC-Kb. *ClCNKB* is supposed to have twelve trans-membrane domains (TM) and intracellular N and cytoplasmic C terminal. It is expressed in the TALH, cortical collecting tubule and distal convoluted tubule (DCT) (the most distal segment of the renal tubule) and regulates the reabsorption of Cl^- in the kidney (5).

Studies have shown that the *CLCNKB* gene is conserved in different species such as the human, the

chimpanzee, Rhesus monkey, dogs, mice, rats, and zebrafish **(6)**. As a consequence, mutations inactivate ClC-Kb, decreasing sodium and chloride tubular reabsorption. Furthermore, the low NaCl and water stimulates the renin-angiotensin-aldosterone system (RAAS)leading to the potassium (K) loss and kidney fibrosis **(5, 7)**.

Growth delay is a frequent medical appearance in patients with BS. The basic mechanisms of growth delay in this disease is not fully clear, but empirical studies has revealed hypokalemia could be a growth hormone (GH) deficiency factor **(8)**. Rats on a low-potassium diet show significant decrease with low concentrations of serum GH and insulin-like growth factor 1 (IGF-1), suggesting that K^+ deficiency (hypo-kalemia) may have a negative-feedback impact on pituitary GH secretion **(3, 9)**. While hypokalemia can play a significant role in growth delay in hypokalemia diseases, for example BS, several patients still have developmental problems after the serum electrolytes normalization.

In the present study, we identified a novel homozygous *CLCNKB* mutation to be the underlying causeof the impairment in an Iranian family with BS. BS caused by *CLCNKB* mutations is inherited in two modes of autosomal recessive and digenic recessive. Human *CLCNKB* gene (MIM 602023) has been located at 1p36.13 chromosome and contains 20 exons and encodes a peptide of 687 residues **(10)**. The proband was a 13-year-old female. Clinical history and biochemical evaluation of the affected patient were indicative of BS. This family is affected by a novel homozygous mutation in exon 19 of *CLCNKB* gene (c.1984insG, p.Gly662GlyfsX12) in the cytoplasmic domain of the protein that is expected to produce a reading frame shift and bring a termination codon at location 674. It is positioned in the similar site of other variant p.Arg595Ter and p.Glu566Argfs*6 from two published cases with the BS that are found in one of the cystathionine-β-synthase (CBS) domains intricated in channel prevalent gating and trafficking can reduce or eliminate normalized conductivity of ClC-Kb **(11-12)**. We speculate that this homozygous mutation can lead to loss-of-function (LOF) of *CLCNKB* gene related to the CBS earlier onset in the proband **(6,8, 13)**.

This was implicated as a pathogenic mutation causing BS. The mutant amino acid sequences are positioned in the highly conserved cytoplasmic domain of CLCNKB that includes a domain in residues 521–687.The C-terminal cytoplasmic domains of CLC are essential for the CLC channel role **(14)**. The position of a homozygous mutation in the exon 19 of *CLCNKB* gene can lead to mRNA helix instability that results in a loss of translation of protein. Furthermore, it is probable that the homozygous mutation interrupts a straight relation to the membrane domain. So far, over 30 *CLCNKB* mutations have been described in BS **(15)**.

Conclusion

Taken together, SAC is capable of alleviating LPS-Here for the first time (to our knowledge) a novel homozygous mutation has been described in *CLCNKB* gene in southwest Iran. The patient demonstrates the importance of molecular analysis in the differential diagnosis and recognition of CBS. Additional surveys of the genetic mechanism of the gene mutation may-present targets for precise treatment in BS patients.

Acknowledgments

None.

Conflict of Interest

None.

References

1. Cunha TDS, Heilberg IP. Bartter syndrome: causes, diagnosis, and treatment. Int J NephrolRenovasc Dis. 2018;11:291-301. [DOI:10.2147/IJNRD.S155397] [PMID] [PMCID]
2. 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. [DOI:10.3389/fped.2018.00153] [PMID] [PMCID]
3. 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(12):F1324-34. [DOI:10.1152/ajprenal.00004.2015] [PMID]
4. Castaño AG, de Nanclares GP, Madariaga L, et al. Genetics of type III Bartter syndrome in Spain, proposed diagnostic algorithm. PLoS One. 2013;8(9):e74673. [DOI:10.1371/journal.pone.0074673] [PMID] [PMCID]
5. Blackburn AT, Miller RK. Modeling congenital kidney diseases in Xenopuslaevis.Dis Model Mech. 2019;12(4).
6. Zelikovic I, Szargel R, Hawash A, et al. A novel mutation in the chloride channel gene, CLCNKB, as a cause of Gitelman and Bartter syndromes. Kidney Int. 2003;63(1):24-32. [DOI:10.1046/j.1523-1755.2003.00730.x] [PMID]
7. Akil I, Ozen S, Kandiloglu AR, Ersoy B. A patient with Bartter syndrome accompanying severe growth hormone deficiency and focal segmental glomerulosclerosis. ClinExpNephrol.

2010;14(3):278-82. [DOI:10.1007/s10157-009-0262-7] [PMID]

8. 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 Med Genet. 2019;20(1):1-6. [DOI:10.1186/s12881-019-0869-9] [PMID] [PMCID]
9. Gil-Peña H, Garcia-Lopez E, Alvarez-Garcia O, et al. Alterations of growth plate and abnormal insulin-like growth factor I metabolism in growth-retarded hypokalemic rats: effect of growth hormone treatment. Am J Physiol Renal Physiol. 2009;297(3):F639-45. [DOI:10.1152/ajprenal.00188.2009] [PMID]
10. Jentsch TJ, Stein V, Weinreich F, Zdebik AA. Molecular structure and physiological function of chloride channels. Physiol Rev. 2002;82(2):503-68. [DOI:10.1152/physrev.00029.2001] [PMID]
11. Nozu K, Fu XJ, Nakanishi K, et al. Molecular analysis of patients with type III Bartter syndrome: picking up large heterozygous deletions with semiquantitative PCR. Pediatr Res. 2007;62(3):364-9. [DOI:10.1203/PDR.0b013e318123fb90] [PMID]
12. Schlingmann KP, Konrad M, Jeck N, et al. Salt wasting and deafness resulting from mutations in two chloride channels. N Engl J Med. 2004;350(13):1314-9. [DOI:10.1056/NEJMoa032843] [PMID]
13. Seys E, Andrini O, Keck M, et al. Clinical and genetic spectrum of Bartter syndrome type 3. J Am SocNephrol. 2017;28(8):2540-52. [DOI:10.1681/ASN.2016101057] [PMID] [PMCID]
14. Seyberth HW. An improved terminology and classification of Bartter-like syndromes. Nat ClinPractNephrol. 2008;4(10):560-7. [DOI:10.1038/ncpneph0912] [PMID]
15. Yu Y, Xu C, Pan X, et al. Identification and functional analysis of novel mutations of the CLCNKB gene in Chinese patients with classic Bartter syndrome. Clin Genet. 2010;77(2):155-62. [DOI:10.1111/j.1399-0004.2009.01288.x] [PMID]

How to Cite This Article:

Mohammadi-asl J, Shahbazian H, Jasemi Zergani F, Kheradmand A R. Identification of a Novel *CLCNKB* Mutation in an Iranian Family with Bartter Syndrome Type 3. J Adv Med Biomed Res. 2022; 30 (139): 185-189.

Download citation:
BibTeX | RIS | EndNote | Medlars | ProCite | Reference Manager | RefWorks

Send citation to:
Mendeley Zotero RefWorks