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CASE REPORT

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Genetic diagnosis of Bartter syndrome in Iranian patients and detection of a novel homozygous *CLCNKB* mutation

Somayeh Takrim Nojehdeh[1] | **Marzieh Mojbafan**[2,3] | **Nakysa Hooman**[4,5] | **Rozita Hoseini**[5] | **Hasan Otukesh**[5]

[1]Dr. Davarnia Genetic Counseling and Diagnosis Center, Imam Reza Hospital, Ardabil, Iran

[2]Department of Medical Genetics, School of Medicine, Iran University of Medical Sciences (IUMS), Tehran, Iran

[3]Department of Medical Genetics, Ali-Asghar Children's Hospital, Tehran, Iran

[4]Clinical research Development Center, Iran University of Medical Sciences, Tehran, Iran

[5]Department of Pediatric Nephrology, Ali-Asghar Children's Hospital, Tehran, Iran

Correspondence
Marzieh Mojbafan, Department of Medical Genetics, School of Medicine, Iran University of Medical Sciences (IUMS), Shahid Hemmat Highway, Tehran, Iran.
Email: marziyeh_mojbafan@yahoo.com

Funding information
Iran University of Medical Sciences

Abstract

An Iranian girl with clinical symptoms of Bartter syndrome like hypokalemia, polyuria, polydipsia, hyponatremia, and hypochloremic alkalosis was referred to us in whom the *CLCNKB* gene was genetically evaluated using Sanger sequencing. A homozygous pathogenic variant of c.1332_1335delCTCT was detected in this patient.

KEYWORDS

Bartter syndrome, CLC-kb, In silico analysis, sanger sequencing

1 | INTRODUCTION

Bartter syndrome, BS, is a rare disease with a prevalence of 1 in 1,000,000 and a heterogeneous group of salt-wasting tubulopathies distinguished by polyuria, polydipsia, hypokalemia, metabolic alkalosis, renal salt loss with normal or low blood pressure and secondary hyperaldosteronism.[1–3] Genotypically, BS divided into at least 6 types results from loss of function mutations in different genes encoding the transporters involved in salt reabsorption at the TAL.[3–5] The type III BS appears in infancy to adolescence and has a wide spectrum of phenotype variability with a predominantly mild presentation.[3,6,7]

CLC-Ka and CLC-Kb belong to a large CLC gene family of chloride channels and are the most voltage-gated Cl^- channels located in the kidney and the inner ear cells.[1,8] CLC-Kb operates as a homodimer associated with the regulatory Barttin subunit leading to reabsorption of Cl^- at thick ascending limb (TAL) of loop of Henle.[8,9] This transmembrane protein is encoded by Cl^- voltage-gated channel Kb (*CLCNKB*) gene [OMIM #602023] which is situated on chromosome 1p36 and includes 20 exons.[3,10] This gene is translated into a 687 amino acid protein[11] which is a plasma membrane protein with distinct regions including 18 α-helices (A-R), 12 transmembrane domains, two cystathionine-β-synthase (CBS) domains, and

intracellular N and C terminal domains.[12] Loss of function mutations in the *CLCNKB* gene leads to BS type III or classic BS with autosomal recessive inheritance form and disruption of electrolyte reabsorption from the renal tubules.[3,13,14]

Since consanguineous marriages are prevalent in Iran,[15] therefore we expect to observe more affected individuals with BS than other parts of the world. In this project, we aim to genetically analyze an Iranian patient who was affected by BS.

2 | MATERIAL AND METHODS

2.1 | Patients

A six-year-old girl affected by BS was referred to Ali Asghar Children Hospital. Informed consent was obtained from the patient and her parents. This study was reviewed and approved by the Iran University of Medical Sciences ethics committee (IR.IUMS.REC.1398.007).

2.2 | Molecular genetic analysis

For mutation screening, genomic DNA was extracted from the peripheral leukocytes from whole blood samples according to the standard procedure.[16] All 20 coding exons and exon-intron boundaries of *CLCNKB* gene were sequenced. All primers are available upon request. Interpretation and fragment analysis were performed using the previously described methods.[17]

2.3 | In silico analysis

After Sanger sequencing, variants were aligned to the RefSeq NM_000085.5 and they were evaluated by different on-line tools such as Mutation Taster and CADD (Combined Annotation Dependent Depletion). Finally, they were interpreted according to the American College of Medical Genetics and Genomics (ACMG) guideline.[18]

3 | RESULTS

Our 6-year-old patient was born to a consanguineous marriage, and her parents were first cousins. She did not show any prenatal symptoms. She was first diagnosed at the age of 4 with hypokalemia, hyponatremia, polyuria, polydipsia, and also metabolic and respiratory alkalosis. She had normal development and did not show any evidence of nephrocalcinosis. she had a positive family history of this disease in her pedigree. (Figure 1). After performing sequencing of all coding exons of the *CLCNKB* gene, the patient showed homozygous variant of c.1332_1335delCTCT (p. Ser445Leufs*33) in the exon 14 of this gene. According to the mutation taster, this variant is a disease causing one, and its PHRED score is 23.9. CADD score is a tool for scoring the deleteriousness of variants. PHRED score greater

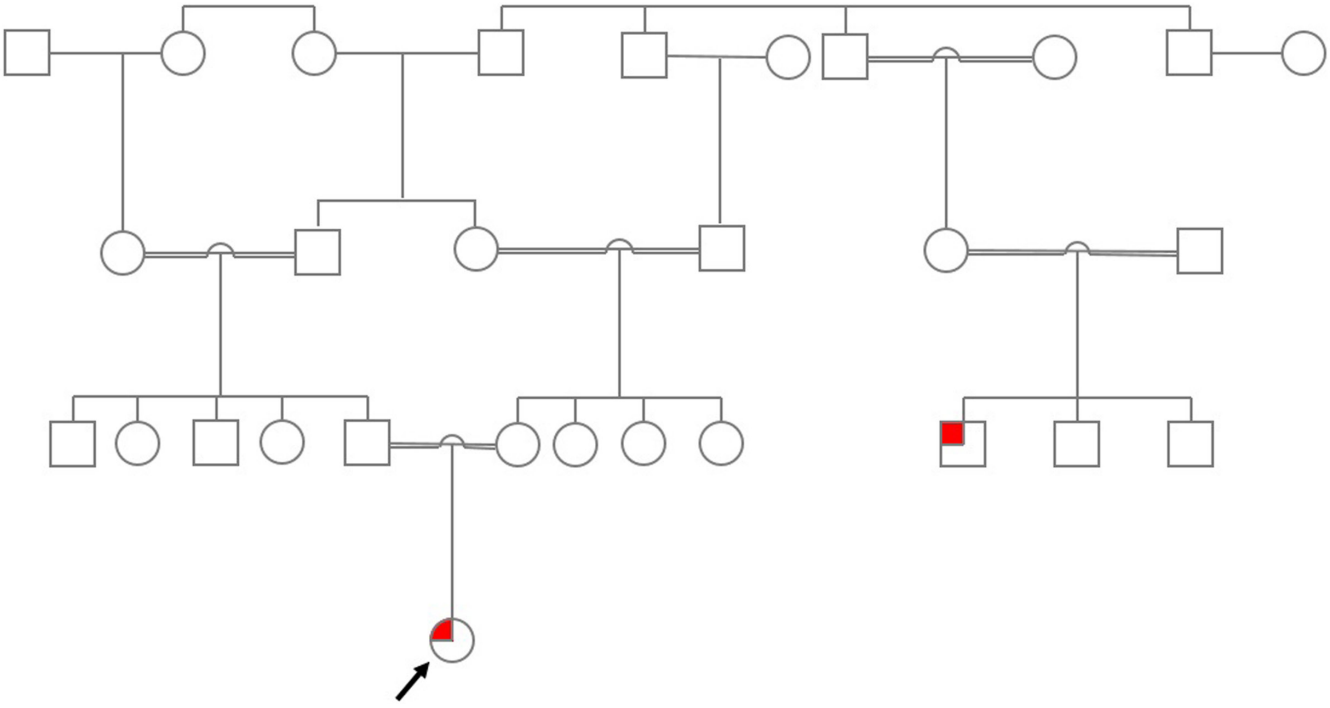

FIGURE 1 Pedigree of our studied patient (arrows display the probands). Colored symbols indicate the affected individuals

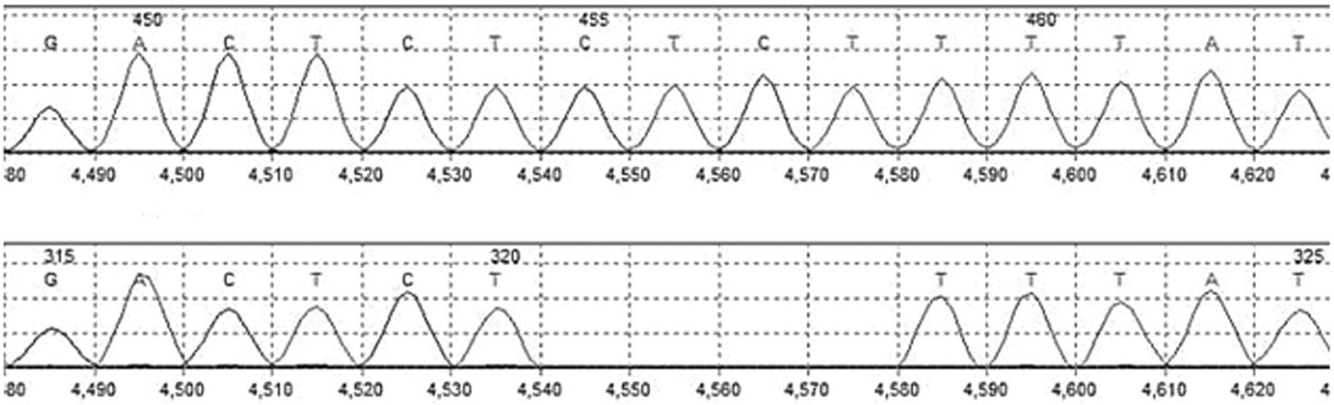

FIGURE 2 Sanger sequencing chromatograms of c.1332_1335delCTCT mutation in the proband. Upper part represents the normal sequence compared with homozygous mutation (down)

than 10 is predicted to be the 10% of the most deleterious variants, and a score of greater or equal to 20 demonstrates the 1% of the most deleterious one. The mutation found in the patient is revealed in Figure 2.

4 | DISCUSSION

Only a few genetic studies of BS have been performed in Iran; Najafi et al. studied a cohort of 17 patients from 15 Iranian families with clinical presentations of BS that twelve patients revealed a large deletion encompassing the whole *CLCNKB* gene which has been identified as a the most frequent mutation of BS type III.[19]

In the present study, we demonstrated a small homozygous deletion mutation (c.1332_1335delCTCT) in the exon 14 of the *CLCNKB* gene. This homozygous deletion caused a frameshift mutation which eliminates the downstream part of the CLC-Kb protein producing a truncated protein, decreases the length of the protein from 687 to 476 amino acids (p.Ser445Leufs*33). Structurally, this mutation is located in the close vicinity to the α-helix N, one of the important regions in gating and ion filtering. Therefore, it can be possible that this truncating mutation reduces the ion conductance.[20] In addition, tow cystathionine-β-synthase domains located at the end of the carboxyl terminus have important roles in the function and/or expression of CLC-Kb, and eliminating these domains due to the mutation may abolish their functions.[1,20,21]

It is noteworthy that this deletion has been previously reported in the compound heterozygote state.[22] This is the first time this mutation c.1332_1335delCTCT is reported in the homozygous state. It is classified as a pathologic one based on the ACMG recommendation because the frameshift mutations are predicted as a very strong proof of pathogenicity (PVS1), the variant has not found in the control population databases such as Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium (PM2). This in-frame deletion changed the length of the protein associated with substitution of the early stop codon (PM4). Several in-silico prediction programs such as CADD, in which the PHRED score is above the cutoff for deleterious variants, interpreted a deleterious effect on the gene (PP3).[18]

5 | CONCLUSION

Since Bartter syndrome is a heterogenous disorder and may have overlapping sign and symptoms with other inherited diseases, using genetic study is a good choice in diagnosis of this disease if it is available.

AUTHOR CONTRIBUTIONS

STN drafted the manuscript. MM conceived the idea, performed and analyzed the experimental data. NH, RH, and HO were involved in the clinical diagnosis of patients. All authors have read and approved the final manuscript.

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CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

Data are available on request from the authors.

CONSENT

Written informed consent was obtained from the patient to publish this report in accordance with the journal's patient consent policy.

ORCID

Somayeh Takrim Nojehdeh https://orcid.org/0000-0003-2799-9961
Marzieh Mojbafan https://orcid.org/0000-0002-9630-3561
Nakysa Hooman https://orcid.org/0000-0002-8494-947X
Rozita Hoseini https://orcid.org/0000-0003-2851-4727
Hasan Otukesh https://orcid.org/0000-0003-2071-517X

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