

Heterozygous Mutations of The Gene for Kir 1.1 (*ROMK*) in Antenatal Bartter Syndrome Presenting with Transient Hyperkalemia, Evolving to a Benign Course

Bartter-like syndrome encompasses a set of inherited renal tubular disorders associated with hypokalemic metabolic alkalosis, renal salt wasting, hyperreninemic hyperaldosteronism, and normal blood pressure. Antenatal Bartter syndrome, a subtype of Bartter-like syndrome, is characterized by polyhydramnios, premature delivery, life-threatening episodes of fever and dehydration during the early weeks of life, growth retardation, hypercalciuria, and early-onset nephrocalcinosis. Mutations in the bumetanide-sensitive Na-K-2Cl cotransporter (*NKCC2*) and ATP-sensitive inwardly rectifying potassium channel (*ROMK*) of the thick ascending limb of Henle's loop have been identified in the antenatal Bartter syndrome. We report the identification of two heterozygous mutations of the gene for Kir 1.1 (*ROMK*) from an antenatal Bartter syndrome patient who presented at birth with mild salt wasting and a biochemical findings that mimicked primary pseudohypoaldosteronism type 1, such as hyperkalemia and hyponatremia, and evolved to a relatively benign course. We have identified amino acid exchanges Arg338Stop and Met357Thr in the gene exon 5 for *ROMK* by PCR and direct sequencing. Both mutations alter the C-terminus of the *ROMK* protein, and can affect channel function.

Key Words : *Bartter's Disease; Potassium Channel; ROMK Protein; Mutation; Pseudohypoaldosteronism*

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INTRODUCTION

In 1962, Bartter and colleagues (1) described a set of new syndrome characterized by hypokalemia, metabolic alkalosis, hyperreninism, hyperaldosteronism with low or normal blood pressure, decreased pressor responsiveness to infused angiotensin II, and hyperplasia of the juxtaglomerular complex. Since this original report, hundreds of Bartter and related syndrome, inherited (autosomal recessive) renal tubular disorders associated with hypokalemic metabolic alkalosis, have been described. These Bartter-like syndromes can be divided into at least three different clinical phenotypes (2, 3): (a) classic Bartter syndrome; (b) Gitelman syndrome; and (c) antenatal (neonatal) Bartter syndrome.

In contrast to classic Bartter syndrome and Gitelman syndrome, the antenatal Bartter syndrome has both the features of renal tubular hypokalemic alkalosis and profound systemic manifestations. Antenatal Bartter syndrome is characterized by polyhydramnios and premature delivery due to intrauterine polyuria. After birth, severe water and salt wasting, hypokalemic metabolic alkalosis, severe hypercalciuria with associated

nephrocalcinosis and osteopenia, and marked growth retardation occur. Systemic manifestations, such as fever, vomiting, and occasional diarrhea, are probably caused by marked stimulation of renal and systemic prostaglandin E₂ production.

Recent studies have identified mutations in four genes as being causative, and have demonstrated genetic heterogeneity in Bartter-like syndrome (4-8): the mutation with loss-of-function in (a) the gene encoding the bumetanide-sensitive Na-K-2Cl cotransporter (*NKCC2*); or (b) the gene encoding an ATP-sensitive inwardly rectifying potassium channel (renal outer medullary K⁺ channel; *ROMK*) of the medullary thick ascending limb (mTAL) of Henle's loop in antenatal Bartter syndrome; (c) the gene encoding a renal chloride channel (*ClC-Kb*) of the mTAL of Henle's loop in classic Bartter syndrome; and (d) the gene encoding the thiazide-sensitive Na-Cl cotransporter (*NCCT*) of the renal distal convoluted tubule in Gitelman syndrome.

In this study, we report the identification of two heterozygous mutations of *ROMK* from antenatal Bartter syndrome presenting with hyponatremia and hyperkalemia, leading to hypokalemia, mild metabolic alkalosis, hypercalciuria and

nephrocalcinosis, and evolving to normalization of biochemical abnormalities except nephrocalcinosis.

MATERIALS AND METHODS

Medical history and laboratory studies of the patient with antenatal Bartter syndrome were reviewed. These studies were approved by the local ethics committees, and the informed consent was obtained from the parents.

Using standard methods, genomic DNA was isolated from 3 mL of EDTA-treated peripheral whole blood of the patient, her parents, and a healthy control. Polymerase chain reaction (PCR) was performed in a 50 μ L volume containing 150 ng of genomic DNA, 1.5 mM MgCl₂, 10 mM Tris (pH 8.6), 50 mM KCl, 0.2 mM dNTP, 30 pmol of each primer, and 2.0 U of *Taq* polymerase (Amersham Pharmacia Biotech Inc., Piscataway, NJ, U.S.A.). Based on the sequence of *ROMK-1*, sets of primer pairs were used to amplify the coding sequence for exon 5 as described previously (6). After the initial denaturation step at 94°C for 5 min, PCR was conducted for 30 cycles with denaturation at 94°C for 45 sec, annealing at 55°C for 45 sec, and extension at 72°C for 45 sec. The reaction was completed with a final elongation step at 72°C for 10 min. Amplified DNA segments were reamplified with the same primer pair and were subjected to direct DNA sequence analysis using an ABI 373A automated DNA sequencer (Applied Biosystems, Foster, CA, U.S.A.). DNA sequences were confirmed by sequencing both strands from the patient, her parents, and a healthy control.

RESULTS

Clinical summary

The female patient was born in the 30th gestational week by cesarean section due to preterm labor and polyhydramnios (birth-weight: 1,450 g). Initially, the patient was hyperkalemic (8.3 mmol/L) and hyponatremic (129 mmol/L), as well as azotemic (BUN 23 mg/dL, creatinine 1.6 mg/dL). After fluid, sodium, and alkali supplementation were initiated, the azotemia, sodium and potassium of the patient were normalized. At two months after birth, she was detected to be hyperkalemic (6.1 mmol/L) and hyponatremic (130 mmol/L). There were no other significant problems until she presented for her routine two-year-old check. At the visit, she was noted to be mild hypokalemic (3.2 mmol/L), metabolic alkalosis (serum bicarbonate 27.5 mmol/L), high renin (22 ng/mL per hour) and aldosterone levels (520 pg/mL), hypercalciuria (9 mg/kg/day), and bilateral nephrocalcinosis. She had no sibling, and there was no family history of abortion or stillbirth. There was no known consanguinity in the family. She was recommended to begin indomethacin treatment. At her next visit for routine three-year-old check, she had a normal serum potassium (3.7 mmol/L), bicarbonate (26 mmol/L), and urine calcium to creatinine ratio (0.1).

Mutational analysis results

Two heterozygous mutations, nonsense and missense, were identified in the patient. The first mutation (C1573T) resulted

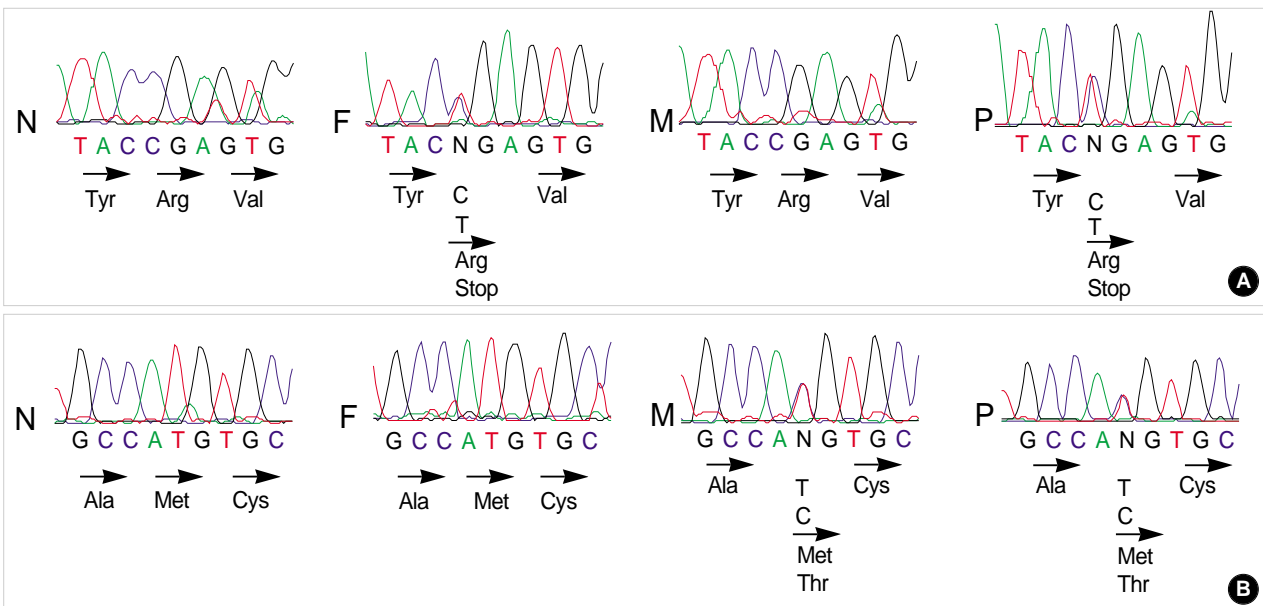


Fig. 1. Identification of mutant alleles for Kir 1.1 gene by genomic sequencing. (A) Corresponding DNA sequence (sense strand) showing the Arg338Stop mutation; (B) Corresponding DNA sequence (sense strand) showing the Met357Thr mutation. N, Healthy control; F, father; M, mother; P, patient

in a stop codon, which was substituted at Arg338 (codon number in *ROMK1*), thereby deleting the terminal 53 residues of the carboxyl tail. The second mutation (T1631C) caused an exchange of Met357 for Thr (Fig. 1). The Arg338Stop and Met357Thr mutations located in the C-terminal intracellular part were transmitted from the father and mother of the patient, respectively, thereby giving rise to a compound heterozygote mutation in the patient.

DISCUSSION

The patient showed typical manifestations of antenatal Bartter syndrome characterized by polyhydramnios, premature delivery, hypercalciuria, and early-onset nephrocalcinosis. However, she presented at birth with mild salt wasting, hyperkalemia, and hyponatremia, suggesting *ROMK* gene mutation. Furthermore, her phenotype has evolved from significant perinatal problems to a relatively benign course at present.

ROMK is expressed in the TAL of Henle's loop where it recycles reabsorbed potassium back to the tubular lumen (K^+ recycling), and in the distal nephron where it contribute to net renal potassium secretion (K^+ secretion). The contribution of the mutation with the loss of *ROMK* function to the antenatal Bartter syndrome can be explained by the impairment of K^+ recycling, resulting in K^+ levels in the lumen that are too low to ensure an adequate function of the Na-K-2Cl cotransporter, producing salt wasting and hypokalemic alkalosis. It has been proposed that the mutation with the loss of *ROMK* function would be expected to result in impaired K^+ secretion in the distal nephron, and explain the clinical picture in some cases of antenatal Bartter syndrome who presented at birth with mild salt wasting and a biochemical findings that mimicked primary pseudohypoaldosteronism type 1, such as hyponatremia, hyperkalemia and metabolic acidosis (3, 5). Furthermore, one might expect to be able to distinguish Bartter syndrome patients with *NKCC2* mutations and unimpaired distal K^+ secretion from those with mutations in *ROMK*, by higher potassium levels (5). However, further study will be required to determine whether the clinical features of the patients with mutations in these two genes can be distinguished clinically.

In human kidney, differential splicing produces five distinct transcripts of *ROMK*. Exon 5 is common to all of these isoforms and encodes the majority of the channel protein (9). All mutations that have been reported so far are located in exon 5 of the *ROMK* gene (5, 6). *ROMK* proteins are the tetramers of four subunits that each have two transmembrane spanning domains (M1 and M2), a pore-forming domain, and intracellular amino and carboxyl termini. The mutations comprise substitutions of the single bases that either lead to single amino acid substitutions or introduce a premature stop codon, and deletions or insertions causing a frameshift. The mutations affect amino acid residues of the transmembrane domains, the

pore-forming domain, the putative ATP-binding domain, as well as the amino and carboxyl termini of *ROMK* channel protein (5, 6).

Mutational analysis of *ROMK* gene in this patient with antenatal Bartter syndrome showing interesting clinical manifestations revealed two mutations Arg338Stop and Met357Thr located in the carboxyl terminus, which were transmitted from the patient's father and mother, respectively, thereby giving rise to a compound heterozygote mutation in the patient. Arg 338Stop and Met357Thr had been previously reported by International Collaborative Study Group for Bartter-like syndromes (6) and Simon et al. (5), respectively. The carboxyl terminus was reported to have a major role in specifying the pore properties of inwardly rectifying potassium channels (10). The nonsense mutation, Arg338Stop, deletes the terminal 53 residues of the carboxyl tail that could alter phosphorylation at the tyrosine kinase site at Tyr337, and cause loss of function in the channel protein (6). The function of the missense mutation, Met357Thr, was suggested to be normal (11). It can help explaining why the patient has been a relatively benign course. However, the functional analysis of the missense mutation, Met357Thr, was done in vitro, and the effect due to regulation by ATP and pH was not completely eliminated. Furthermore, her significant perinatal problems strongly suggest that the function of Met357Thr can be abnormal in vivo, and regulated by some factors in vitro. Therefore, the more extensive functional analyses of these mutant proteins will be necessary to prove their causative role and to explain interesting clinical course in the patient.

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