Novel SLC12A1 (NKCC2) Mutations in Two Families with Bartter Syndrome Type 1

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Abstract. Bartter syndrome (BS) type 1, also referred to antenatal BS, is a genetic tubulopathy with hypokalemic metabolic alkalosis and prenatal onset of polyuria leading to polyhydramnios. It has been shown that BS type 1 is caused by mutations in the SLC12A1 gene encoding bumetanide-sensitive Na-K-2Cl–cotransporter (NKCC2). We had the opportunity to care for two unrelated Japanese patients of BS type 1 with typical manifestations including polyhydramnios, prematurity, hypokalemia, alkalosis, and infantile-onset nephrocalcinosis. Analysis of the SLC12A1 gene demonstrated four novel mutations: N117X, G257S, D792fs and N984fs. N117X mutation is expected to abolish most of the NKCC2 protein, whereas G257S, which is evolutionary conserved, resides in the third transmembrane domain. The latter two frameshift mutations reside in the intracellular C-terminal domain, which illustrates the importance of this domain for the NKCC2 function. In conclusion, we found four novel SLC12A1 mutations in two BS type 1 patients. Development of effective therapy for hypercalciuria is mandatory to prevent nephrocalcinosis and resultant renal failure.

Key words: Antenatal Bartter syndrome, Bumetanide-sensitive Na-K-2Cl–cotransporter, Neonatal Bartter syndrome, Nephrocalcinosis

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BARTTER syndrome (BS) is one of the most frequent forms of inherited tubulopathy, characterized by urinary potassium loss and metabolic alkalosis [1, 2]. Knowledge of the molecular basis for BS has accelerated our understanding of this condition, and the classification according to the responsible genes has since prevailed. Of these, BS type 1 [OMIM#601678], antenatal (or neonatal) BS (ABS), is caused by homozygous or compound heterozygous mutations in the SLC12A1 gene encoding bumetanide-sensitive sodium-potassium-chloride cotransporter-2 (NKCC2) [3, 4]. In ABS, abnormality begins in utero with marked fetal polyuria resulting in polyhydramnios and premature delivery. Here we report two unrelated ABS patients with novel SLC12A1 mutations.

Case presentation

Patient 1

A female baby was born to non-consanguineous healthy Japanese parents with her weight 2,340 g at 35
5/7 wk of gestational age. She was delivered by caesarean section because of fetal distress. Apgar score was 9 at 5 min. Her pregnancy course was complicated by polyhydramnios since 27th wk of gestation which warranted amniocentesis 9 times. Fetal urine production rate was estimated as much as 60 mL/h. Coupled with elevated Cl\(^-\) (117 mEq/L) and aldosterone (2,470 pg/mL) levels in the amniotic fluid, the diagnosis of ABS had been anticipated. Polyuria became apparent 6 hrs after birth, with urinary volume ranging from 150–450 mL/kg/day, which necessitated large amounts of transfusion. Subsequently, hypokalemia (2.9 mEq/L), hypocalemia (4.2 mg/dL) and hypomagnesemia (1.1 mg/dL) developed. Plasma aldosterone level at the day of birth was revealed to be elevated (1,300 pg/mL [normal range for neonates: 141–1,112 pg/mL]), as well as PRA (58 ng/mL/hr [normal range for neonates: <17.5 ng/mL/hr]). Oral indomethacin, with relatively small dose up to 1.0 mg (0.33 mg/kg) a day, was effective in reducing the amount of transfusion and in maintaining the electrolyte concentrations. Spironolactone (2 mg/kg/day) was tried, but had to be withdrawn because of its adverse effect of hypertriglyceridemia. Until 6 years of age, she grew normally on indomethacin administration (0.9–1.4 mg/kg/day) alone. Her serum potassium level has gradually declined (0.6–1.2 mg/mg of creatinine), which led to the appearance of nephrocalcinosis on ultrasound at 1.5 years of age.

**Patient 2**

The second patient is a female who was born at 33 3/7 week with her weight 1,918 g. Asphyxia was not observed. Her mother developed hyperthyroidism at 15th wk of gestation and was prescribed anti-thyroidal agents. Polyhydramnios was noticed at around the 20th wk of gestation which warranted frequent amniocentesis. After a premature rupture of the membrane, the baby was born through vaginal delivery. She was found to have polyuria greater than 75 mL/kg/day on the day of birth. Her urine volume had increased to 270 mL/kg/day, which led to 16% weight loss at the 9th day. Hypokalemia (2.8 mEq/L), as well as hyponatremia (133 mEq/L) and hypochloremia (84 mEq/L) developed at the 6th day, which needed correction by electrolyte transfusion. Hypomagnesemia was not recognized. Combination therapy with indomethacin (0.1 mg/kg/day) and spironolactone (1.4 ng/kg/day) was started during the 4th wk of life followed by reduction of urine volume and remarkable weight gain. Treatment for transient hyperthyroidism was also needed with anti-thyroidal agent and iodide, which could be withdrawn within 3 wks. Hypercalciuria was evident, and nephrocalcinosis was found by ultrasound scanning performed at 87th postnatal day.

**Method**

Genomic DNA was prepared from white blood cells of both patients and their parents using standard techniques. The SLC12A1 gene was analyzed by PCR-direct sequencing as previously described [5]. The primer sequences were designed according to the report of Simon et al. [3]. All nucleotide variations observed were confirmed from independent PCR. DNA samples from 30 healthy, unrelated Japanese individuals served as normal controls.

In the family of patient 1, linkage analysis using microsatellite markers around SLC12A1 locus (15q) was performed to confirm the parent-child relationship. ABI PRIMS Linkage Mapping Sets v2.5 Panel 22, which includes 7 markers on chromosome 15, was used [Applied Biosystems, Foster City, CA, U.S.A.]. DNA samples from patient 1 and her parents were amplified by PCR according to the manufacturer’s protocol, and the length of each marker was visualized by gel-electrophoresis.

With all the experiments above, written informed consent was obtained from the parents.

**Results**

In patient 1, heterozygous mutation of 367insT (in exon 1) was found (Fig. 1A). This insertion was expected to introduce a nonsense mutation in the immediate downstream codon (N117X). This insertion was also identified in her mother. In addition, patient 1 had g788a (G257S) mutation in exon 5 (Fig. 1A). This substitution was not found in either of her parents and in normal controls. The results of the linkage analysis performed in her family did not deny paternity and maternity (data not shown).

In patient 2, heterozygous mutations of 2393-
2394del GA (in exon 18, introducing a frameshift at E792 and causing nonsense codon 4 nucleotides downstream) and 2971-2974del CAAA (in exon 23, introducing a frameshift at N984 and causing nonsense codon 26 nucleotides downstream) were found (Fig. 1B). The former mutation was also identified in her mother, whereas the latter was found in her father.

**Discussion**

The hallmark of ABS is polyhydramnios and prematurity, followed by postnatal polyuria and hypercalciuria [1, 2]. To date, three different genes are found to be responsible for this condition: the SLC12A1 (NKCC2) gene which resides in chromosome 15q (type 1 BS), the ROMK (KCNJ1) gene [6] located on 11q24-25 (type 2, OMIM#241200), and BSND gene [7], which encodes the common accessory β-subunit barttin for ClC-Ka and ClC-Kb chloride channels (type 4, OMIM#602522).

There have been reported about 30 mutations in SLC12A1 gene (Fig. 2), and this is the second mutational report from Japanese patients. Our results indicate that diverse mutational spectrum, except for founder mutation (W625X) in Costa-Rican population [8], holds true also in Japanese population. Despite mutational diversity, the phenotypes of ABS have been considered to be rather uniform. The clinical courses of the two patients described here were also typical for ABS. However, phenotypic variability among ABS patients with SLC12A1 mutations has been observed, such as absence of hypokalemia and/or metabolic alkalosis in the first year of life and presence of metabolic acidosis or hypernatremia [9]. In addition, a recent report of late-onset manifestation of ABS due to residual NKCC2 function indicates that genotype-phenotype relationship is maintained in ABS patients with SLC12A1 mutations [10]. In this context, the two deletion mutations in the C-terminal domain found in our patient 2 suggest that this region is crucial for the transporting function of NKCC2.

One missense mutation found in patient 1 (G275S) was not present in her parents. Results of the linkage
analysis in her family did not deny the parent-child relationship, which also suggested that labeling error of samples was unlikely. It may be the case that it had arisen de novo, or that her father has somatic mosaicism. Alternatively, there is a possibility that G275S mutation resides in the maternal allele (the same allele with N117X) in patient 1. Further study is warranted to clarify these issues. Glycine at position 275 has been evolutionary conserved in the predicted 3rd transmembrane domain of the NKCC2 protein [11]. In addition, we could not find G275S substitution in the SNP databases either in global (dbSNP) and Japanese populations (JSNP, ref 12). Accordingly, we think that this substitution is not a polymorphism, but indeed a mutation.

Both of our two patients showed overt hypercalciuria. However, the advent of nephrocalcinosis was delayed to 1.5 years of age in patient 1, whereas it was as early as 3 months in patient 2. We do not have an explanation for this discrepancy, but co-existence of neonatal hyperthyroidism in patient 2 may facilitate the hypercalciuria via enhanced bone absorption. Although nephrocalcinosis may lead to renal failure [13], we have no treatment of choice at present, which is one of the major problems to be resolved hereafter.

In conclusion, we reported four novel SLC12A1 gene mutations in two unrelated Japanese patients with typical ABS. Molecular analysis was helpful in establishing definite diagnosis.

References


