Large Deletion of the 5' End of the ROMK1 Gene Causes Antenatal Bartter Syndrome

DELPHINE FELDMANN,* JEAN-LUC ALESSANDRI,‡ and GEORGES DESCHÊNES†
*Laboratory of Biochemistry and ‡Department of Pediatric Nephrology, Armand Trousseau Hospital, Paris, France; and †Department of Pediatric Intensive Care, Félix Guyon Hospital, Saint Denis, La Réunion, France.

Abstract. Mutations in exon 5 of the ROMK1 gene (KCNJ1) have recently been observed in antenatal Bartter syndrome patients. This study describes a homozygous deletion removing KCNJ1 exons 1 and 2 observed in a consanguineous family with antenatal Bartter syndrome. Absence of the untranslated exon 1 led to the deletion of transcription elements located in this exon that may cause the disease. Deletion of exon 1 transcription elements should lead to the absence of hROMK2-K5 transcripts, whereas hROMK1 transcripts should normally be transcribed. Consequently, probably only hROMK2-K5 transcripts are expressed in the medullary thick ascending limb of Henle.

Antenatal Bartter syndrome (ABS) is an inherited tubular disorder beginning in utero and resulting in polyhydramnios and premature delivery (1). Affected neonates have severe salt loss, moderate metabolic alkalosis and hypokalemia, renin and aldosterone hypersecretion, and severe hypercalcuiuria with early nephrocalcinosis. A disorder of the medullary thick ascending limb of Henle has been demonstrated. Recent molecular studies have shown that mutations in the genes encoding the Na-K-2Cl cotransporter (2,3), the chloride channel CLC-NKB (4), and the potassium channel ROMK have been detected in ABS patients (5,6).

The ROMK gene (locus KCNJ1) encodes a K⁺ channel that belongs to the family of the inwardly rectifying K⁺ channel (Kir family) (7). Kir family channels comprise two predicted transmembrane domains flanking an H5-like region which forms part of the channel pore. At least five distinct hROMK transcripts have been identified in the human kidney (8,9). They encode three peptides that differ in their N-terminal extensions (hROM-K1, -K2, -K3). Exon 5 of KCNJ1 is common to all of the transcripts and encodes most of the channel protein. The mutations described previously in ABS patients have been located in exon 5 (5,6). We report a large deletion of exons 1 and 2 within KCNJ1 in a consanguineous ABS family. In this family, previous linkage analysis with microsatellite markers in 11q24–25 and 15q15–21 suggested a linkage between KCNJ1 and ABS and allowed exclusion of gene NKCC2 encoding the Na-K-2Cl cotransporter (6).

Materials and Methods

Patients

Clinical analysis of three ABS patients from the same family was made from the records of Armand Trousseau and Félix -Guyon hospitals.

Mutation Analysis

DNA was extracted from whole blood lymphocytes of all family members IV and V, except IV-4, V-2, and V-7. The five exons and the exon-intron boundaries were amplified using the sets of primers described previously (5,6). Amplification products were separated in Nusieve 3:1 agarose (FMC BioProducts, Rockland, ME) and studied by single-strand conformation polymorphism (SSCP) analysis. SSCP was carried out on a GenePhor Electrophoresis Unit (Pharmacia Biotech) using the GeneGen Clean 15/24 according to the manufacturer's instructions (Pharmacia Biotech).

Southern Blotting

Genomic DNA was digested with BamHI. The digested products were analyzed by electrophoresis on 0.8% agarose gels (17 h at 44 V). Alkaline transfer to nylon membrane Genescreen+ (New England Nuclear) was performed over 4 h. The membrane was then hybridized with P³²-labeled PCR KCNJ1 probes and human Cot-1 DNA (Life Technologies).

Results

Clinical Phenotype

A consanguineous family of Indian extraction in which three neonates presented with ABS is reported (Figure 1). Patient V-1 was born at 31 wk after a pregnancy marked by polyhydramnios from the 28th week. Polyuria and sodium loss necessitated parenteral infusion to maintain normal hydration during the first 6 wk of life. At 1 yr of age, he suffered from failure to thrive (−3 SD), polyuria (230 ml/kg per d), and repeated unexplained episodes of fever. Nephrocalcinosis and renal lithiasis at 2.5 yr of age were associated with hypercalcuiuria (12 mg/kg per d). Mild hypokalemia (3.4 mmol/L),...
metabolic alkalosis (27 mmol/L), isosthenuria (270 mosmol/L), and renin hypersecretion also occurred at the same age. Indomethacin was given until 5 yr of age resulting in growth catch-up and decrease in polyuria. Patient V-7 died on the first day of life. The pregnancy was marked by polyhydramnios from the 20th week and premature delivery at 24 wk. Patient V-10 was born prematurely at 32 wk after a pregnancy marked by polyhydramnios. Moderate hypokalemia (3.5 mmol/L), renin hypersecretion (50 pg/ml), aldosteronism (1900 pg/ml), and hypercalciuria (urinary calcium/creatinine = 2.44 mmol/mmol) were identified at this time.

**Mutation Analysis**

Amplification of exons 1, 2, and 3 is presented in Figure 2. Exons 1 and 2 could not be amplified in the two affected patients. Amplification of exons 1 and 2 of all of the other family members gave a product of the expected size. Amplification of exons 3, 4, and 5 gave a product of the expected size for all of the samples studied. Amplification products of exons 3, 4, and 5 were analyzed by SSCP. Normal SSCP patterns were observed for all of the family samples studied. Amplification of exons 1 and 2 in 50 unaffected subjects gave a product of the expected size.

**Southern Blot Analysis**

*KCNJ1* sequence (GenBank accession no. U65406) revealed seven restriction sites for *BamHI* (Figure 3). The 12.6-kb fragment contains exons 4 and 5, and the 3.6-kb fragment contains exons 1 and 2. In the two patients, the 3.6-kb fragment was absent and no additional fragments were observed, thus confirming the homozygous deletion of exons 1 and 2 (Figure 4).

**Discussion**

Two recent studies have shown that mutations in *KCNJ1* can induce ABS (5,6). The 18 mutations described previously in ABS patients are located in *KCNJ1* exon 5. By contrast, we observed a large homozygous deletion in the 5' region of *KCNJ1* in patients of a consanguineous family. According to genealogical data and mutation analysis, parents are heterozygous for the deletion. This deletion of at least 884 bp removed all of exons 1 and 2 and intron 1. Exon 3 distant from exon 2 of 15,633 bp was not affected. The cosegregation of the mutation with the disease and the absence of another alteration in *KCNJ1* suggest that this mutation causes ABS in the family reported. Previous cloning of ROMK cDNA (8–10) has revealed the existence of at least five distinct transcripts in the human kidney (*hROMK1-K5*). These transcripts are generated from only one gene by an alternative splicing mechanism and differ by their 5' region. *hROMK1* results in the combination of exons 4 and 5. The other four transcripts contain exons 1 and 5 and variable combination of exons 2 and 3. Transcription could be initiated from either the 5' end of exon 1 or exon 4.
weight proteins in addition to proteins of the expected size on the rat kidney nephron, which revealed high molecular channel. This is supported by immunoblot analysis performed that all hROMK isoforms are necessary to form a functional K

5, contains exons 4 and exons 1 and 2 and intron 1 were used. The upper fragment (12.6 kb) corresponding to exon 4 and a KCNJ1 (13).

The most convincing hypothesis to explain expression of ABS in the family reported is that only hROMK2-K5 transcripts are expressed in medullary thick ascending limb of Henle, whereas hROMK1 transcripts are located in another part of the nephron. This hypothesis is supported by a previous report on rat ROMK transcripts. Indeed, rROMK are expressed in different parts of the nephron (11). However, we cannot exclude fetal and postnatal regulation of hROMK expression. Indeed, regulation of ROMK during gestation has been observed in the pregnant rat uterus (12). A low level of hROMK1 expression at an early stage of life would be insufficient to balance the lack of hROMK2-3 expression. This could explain the improvement in the disease with age. Another hypothesis is that all hROMK isoforms are necessary to form a functional K channel. This is supported by immunoblot analysis performed on the rat kidney nephron, which revealed high molecular weight proteins in addition to proteins of the expected size (13).

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References

Figure 4. KCNJ1 Southern blot results. The identity of the template DNA is indicated at the top of each lane. A KCNJ1 PCR probe corresponding to exon 4 and a KCNJ1 PCR probe corresponding to exons 1 and 2 and intron 1 were used. The upper fragment (12.6 kb) contains exons 4 and 5, and the lower fragment (3.6 kb) contains exons 1 and 2.