

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

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**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 hypercalciuria 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<sup>+</sup> channel that belongs to the family of the inwardly rectifying K<sup>+</sup> 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 conformational polymorphism (SSCP) analysis. SSCP was carried out on a GenePhor Electrophoresis Unit (Pharmacia Biotech) using the GeneGel Clean 15/24 according to the manufacturer's instructions (Pharmacia Biotech).

### Southern Blotting

Genomic DNA was digested with *Bam*HI. The digested products were analyzed by electrophoresis on 0.8% agarose gels (17 h at 44 V). Alkaline transfer to nylon membrane Genescreen<sup>+</sup> (New England Nuclear) was performed over 4 h. The membrane was then hybridized with P<sup>32</sup>-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 hypercalciuria (12 mg/kg per d). Mild hypokalemia (3.4 mmol/L),

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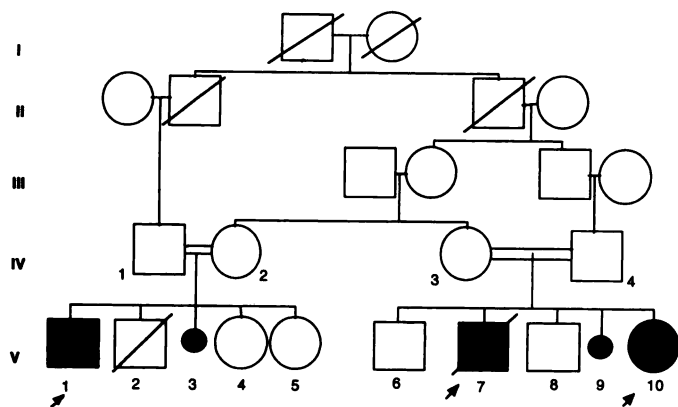


Figure 1. Family pedigree. Filled symbols indicate affected individuals. Index cases are indicated by an arrow.

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 *Bam*HI (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

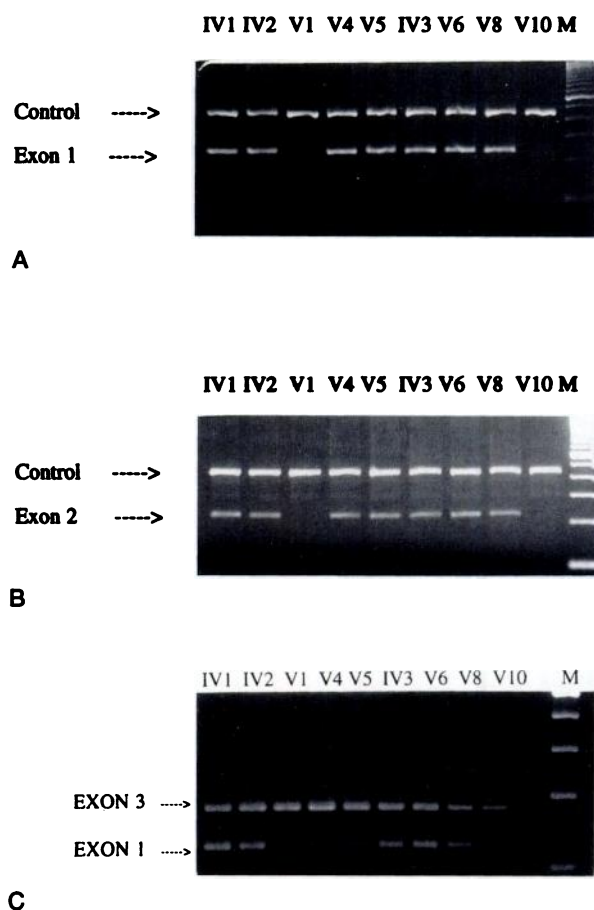


Figure 2. PCR amplification of *KCNJ1* in a family with antenatal Bartter syndrome. The identity of the template DNA is indicated at the top of each lane. M, molecular weight marker. (A) Amplification of *KCNJ1* exon 1. (B) Amplification of *KCNJ1* exon 2. (C) Amplification of *KCNJ1* exons 3 and 1. Primers specific for intron 19 of an unrelated gene, *CFTR*, were included in the reaction as a positive control to ensure that the absence of product was not simply due to PCR failure.

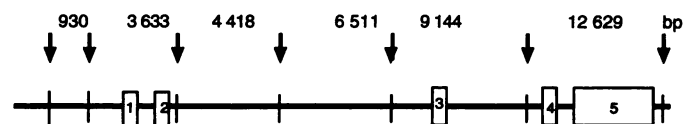
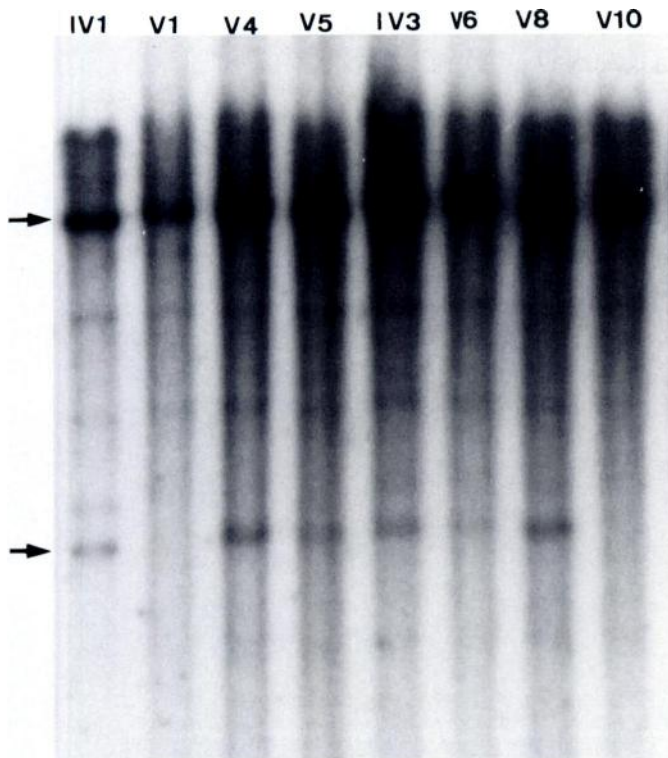


Figure 3. Schematic structure of *KCNJ1*. Exons are depicted by open boxes; introns are represented by lines. *Bam*HI restriction sites are indicated by an arrow.

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



**Figure 4.** *KCNJI* Southern blot results. The identity of the template DNA is indicated at the top of each lane. A *KCNJI* PCR probe corresponding to exon 4 and a *KCNJI* 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.

(10). The deletion of exon 1 transcription elements observed in patients should lead to the absence of *hROMK2-K5* transcripts, whereas *hROMK1* transcripts should be present. Therefore, neither the *hROMK2* nor the *hROMK3* peptide encoded by the *hROMK2-K5* transcripts should be present in these patients, while the *hROMK1* isoform would normally be translated from the *hROMK1* transcript (9).

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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