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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 transcripted. 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^+ 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).

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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$^+$ (New England Nuclear) was performed over 4 h. The membrane was then hybridized with P^{32}-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),

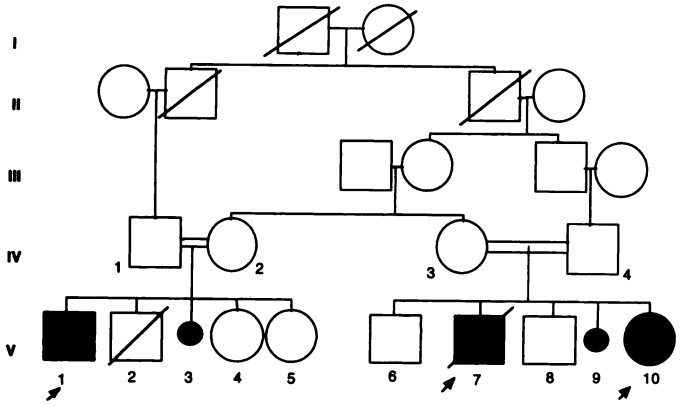

Figure 1. Family pedigree. Filled symbols indicate affected individuals. Index cases are indicated by an arrow.

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).

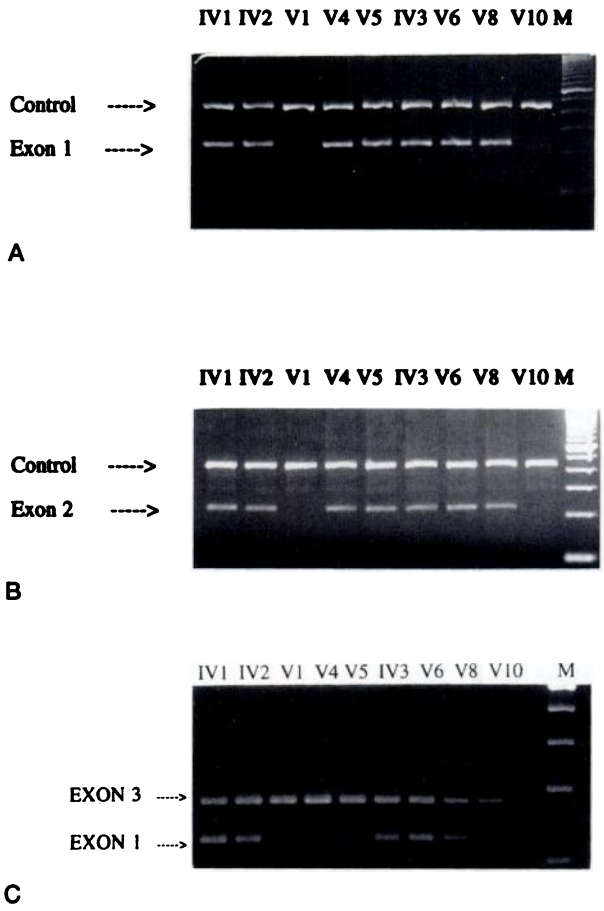

Figure 2. PCR amplification of *KCNJ1* in a family with antenatal Bartter syndrome. The identity of the template DNA is indicated at the top of each lane. M, molecular weight marker. (A) Amplification of *KCNJ1* exon 1. (B) Amplification of *KCNJ1* exon 2. (C) Amplification of *KCNJ1* exons 3 and 1. 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.

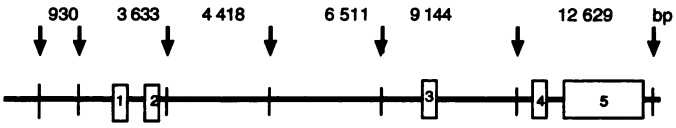

Figure 3. Schematic structure of *KCNJ1*. Exons are depicted by open boxes; introns are represented by lines. *Bam*HI restriction sites are indicated by an arrow.

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

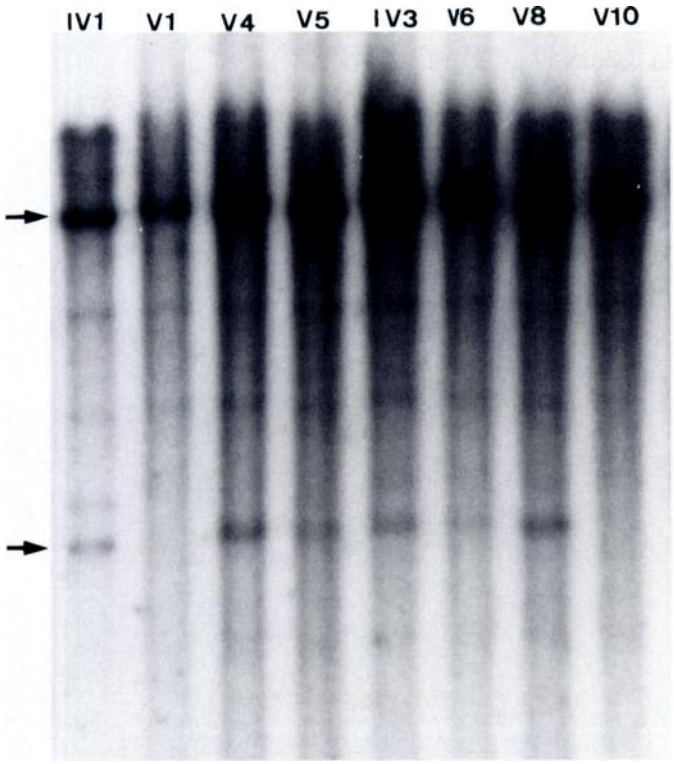

Figure 4. KCNJ1 Southern blot results. The identity of the template DNA is indicated at the top of each lane. A *KCNJ1* PCR probe corresponding to exon 4 and a *KCNJ1* PCR probe corresponding to exons 1 and 2 and intron 1 were used. The upper fragment (12.6 kb) contains exons 4 and 5, and the lower fragment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

(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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References

1. Proesmans W: Bartter syndrome and its neonatal variant. *Eur J Pediatr* 156: 669–679, 1997
2. Simon DB, Karet FE, Hamdan JM, DiPietro A, Sanjad SA, Lifton RP: Bartter's syndrome, hypokalaemic alkalosis with hypercalciuria, is caused by mutations in the Na-K-2Cl cotransporter NKCC2. *Nat Genet* 13: 183–188, 1996
3. Kurtz CL, Karolyi L, Seyberth HW, Koch MC, Vargas R, Feldmann D, Vollmer M, Knoers NV, Madrigal G, Guay-Woodford LM: A common NKCC2 mutation in Costa Rican Bartter's syndrome patients: Evidence for a founder effect. *J Am Soc Nephrol* 8: 1706–1711, 1997
4. Simon DB, Bindra RS, Mansfield TA, Nelson-Williams C, Mendonca E, Stone R, Schurman S, Nayir A, Alpay H, Bakkaloglu A, Rodriguez-Soriano J, Morales JM, Sanjad SA, Taylor CM, Pilz D, Brem A, Trachtman H, Griswold W, Richard GA, John E, Lifton RP: Mutations in the chloride channel gene, CLCNKB, cause Bartter's syndrome type III. *Nat Genet* 17: 171–178, 1997
5. Simon DB, Karet FE, Rodriguez-Soriano J, Hamdan JH, DiPietro A, Trachtman H, Sanjad SA, Lifton RP: Genetic heterogeneity of Bartter's syndrome revealed by mutations in the K+ channel, ROMK. *Nat Genet* 14: 152–156, 1996
6. International Collaborative Study Group for Bartter-Like Syndromes: Mutations in the gene encoding the inwardly-rectifying renal potassium channel, ROMK, cause the antenatal variant of Bartter syndrome: Evidence for genetic heterogeneity. *Hum Mol Genet* 6: 17–26, 1997
7. Wang WH, Hebert SC, Giebisch G: Renal K+ channels: Structure and function. *Annu Rev Physiol* 59: 413–436, 1997
8. Yano H, Philipson LH, Kugler JL, Tokuyama Y, Davis EM, Le Beau MM, Nelson DJ, Bell GI, Takeda J: Alternative splicing of human inwardly rectifying K+ channel ROMK1 mRNA. *Mol Pharmacol* 45: 854–860, 1994
9. Shuck ME, Bock JH, Benjamin CW, Tsai TD, Lee KS, Slightom JL, Bienkowski MJ: Cloning and characterization of multiple forms of the human kidney ROM-K potassium channel. *J Biol Chem* 30: 24261–24270, 1994
10. Bock JH, Shuck ME, Benjamin CW, Chee M, Bienkowski MJ, Slightom JL: Nucleotide sequence analysis of the human KCNJ1 potassium channel locus. *Gene* 188: 9–16, 1997
11. Boim MA, Ho K, Shuck ME, Bienkowski MJ, Block JH, Slightom JL, Yang Y, Brenner BM, Hebert SC: ROMK inwardly rectifying ATP-sensitive K+ channel. II. Cloning and distribution of alternative forms. *Am J Physiol* 268: F1132–F1140, 1995
12. Lundgren DW, Moore JJ, Chang SM, Collins PL, Chang AS: Gestational changes in the uterine expression of an inwardly rectifying K+ channel, ROMK. *Proc Soc Exp Biol Med* 216: 57–64, 1997
13. Xu JZ, Hall AE, Peterson LN, Bienkowski MJ, Eessalu TE, Hebert SC: Localization of the ROMK protein on apical membranes of rat kidney nephron segments. *Am J Physiol* 273: F739–F748, 1997