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Epilepsy, Ataxia, Sensorineural Deafness, Tubulopathy, and *KCNJ10* Mutations

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Abstract

BACKGROUND—Five children from two consanguineous families presented with epilepsy beginning in infancy and severe ataxia, moderate sensorineural deafness, and a renal salt-losing tubulopathy with normotensive hypokalemic metabolic alkalosis. We investigated the genetic basis of this autosomal recessive disease, which we call the EAST syndrome (the presence of epilepsy, ataxia, sensorineural deafness, and tubulopathy).

METHODS—Whole-genome linkage analysis was performed in the four affected children in one of the families. Newly identified mutations in a potassium-channel gene were evaluated with the use of a heterologous expression system. Protein expression and function were further investigated in genetically modified mice.

RESULTS—Linkage analysis identified a single significant locus on chromosome 1q23.2 with a lod score of 4.98. This region contained the *KCNJ10* gene, which encodes a potassium channel expressed in the brain, inner ear, and kidney. Sequencing of this candidate gene revealed homozygous missense mutations in affected persons in both families. These mutations, when expressed heterologously in xenopus oocytes, caused significant and specific decreases in potassium currents. Mice with *Kcnj10* deletions became dehydrated, with definitive evidence of renal salt wasting.

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CONCLUSIONS—Mutations in *KCNJ10* cause a specific disorder, consisting of epilepsy, ataxia, sensorineural deafness, and tubulopathy. Our findings indicate that *KCNJ10* plays a major role in renal salt handling and, hence, possibly also in blood-pressure maintenance and its regulation.

The study of molecular defects in rare inherited renal tubular diseases has substantially advanced both our understanding of renal salt and water handling and the management of common disorders such as systemic hypertension.^{1–7} Two well-known disorders of renal tubular salt handling, Bartter's syndrome and the Gitelman syndrome, are characterized by polyuria and normotensive hypokalemic metabolic alkalosis. We describe five children with similar clinical findings, as well as infantile-onset seizures, ataxia, and sensorineural deafness. We determined the genetic basis of this disease and the pathophysiological mechanism accounting for its seemingly divergent clinical manifestations.

METHODS

GENOTYPING AND LINKAGE ANALYSIS

Genetic studies were approved by the Institute of Child Health–Great Ormond Street Hospital Research Ethics Committee, and the parents provided written informed consent. Genotypes from DNA of the four affected children in Family 1 and the parents of the three affected siblings within this pedigree (Fig. 1A) were generated with the use of single-nucleotide polymorphism (SNP) chip arrays (GeneChip Human Mapping 10K Array Xba142 2.0, Affymetrix) according to the manufacturer's recommendations. Genotypes were examined with the use of a multipoint parametric linkage analysis and haplotype reconstruction performed with SimWalk (version 2.91) for an autosomal recessive model with complete penetrance and a disease allele frequency of 0.001 (deCode SNP map with Asian allele frequencies).⁸ The data were formatted with Mega2 (version 4.0) through ALOHOMORA (version 0.30, Win32).^{9,10} Mendelian inconsistencies were checked with the use of PedCheck (version 1.1); unlikely genotypes were filtered with the use of Merlin (version 1.1, alpha 3).^{11,12} The SimWalk haplotype output files were visualized with HaploPainter.¹³

SEQUENCING

We sequenced the complete coding region and splice sites of *KCNJ10*, a gene encoding a potassium channel expressed in the brain, inner ear, and kidney, in all the affected children and their unaffected parents, as previously reported.¹⁴ The presence of sequence variations was checked in at least 100 ethnically matched control alleles.

ELECTROPHYSIOLOGICAL STUDIES

Electrophysiological measurements were performed with the use of a classic two-electrode voltage-clamp microelectrode approach. Heterologous expression of mutant and wild-type *KCNJ10* in *Xenopus laevis* oocytes was produced as previously reported.¹⁵ Studies conformed to relevant U.K. Home Office regulations. In short, oocytes were injected with 4 to 20 ng of complementary RNA (cRNA), transcribed with the use of mMessage-Machine (Applied Biosystems), from human *KCNJ10* cloned into the vector pTLB. Measurements were performed in 20 mM of potassium chloride at –80 mV in at least four batches of oocytes. *KCNJ10*-specific currents in the injected oocytes, as compared with uninjected oocytes, were confirmed by means of blockade with barium.

KCNJ10 KNOCKOUT MICE

Kcnj10 knockout mice were generated as previously described¹⁶ and were provided by Drs. Clemens Neusch and Frank Kirchhoff, Max Planck Institute for Experimental Medicine,

Göttingen, Germany. The experiments were approved by the local councils for animal care and conducted according to German regulations governing animal care.

Urine was collected from the mice when they were 3 days old and was analyzed as previously reported.¹⁷ Urine electrolytes were measured by Katja Huggel and Dr. François Verrey, University of Zürich, Zurich, Switzerland, with the use of ion chromatography (Metrohm).

Immunolocalization studies of kidneys from 12-week-old C57BL/6 mice (Charles River) were performed according to published methods.¹⁸ Incubation and imaging with the use of primary antibodies to KCNJ10 (Alomone), aquaporin-2 (St. Cruz), calbindin (Sigma), NKCC2,¹⁹ and NCC²⁰ were performed according to standard procedures.

STATISTICAL ANALYSIS

Electrophysiological in vitro experiments and mouse urinalyses were interpreted with the use of an unpaired, two-sided Student's t-test comparing effect and appropriate controls. Data are reported as means and standard error of the mean. A P value of less than 0.05 was considered to indicate statistical significance.

RESULTS

CLINICAL FINDINGS

We identified five children from two consanguineous families (Fig. 1A) who had the clinical features of epilepsy, ataxia, sensorineural deafness, and a renal salt-losing tubulopathy. All other members of these families were clinically unaffected. None of the five children (four in Family 1 [Patients 1-1, 1-2, 1-3, and 1-4] and one in Family 2 [Patient 2-1]) had been born prematurely.

All affected patients initially presented with generalized tonic-clonic seizures in infancy (Table 1). These seizures were easily controlled with a broad-spectrum anticonvulsant agent, but focal seizures subsequently occurred in Patient 1-2. Routine electroencephalographic recordings in Patients 1-1 and 1-2 were normal at the ages of 7 and 8 years, respectively. All five patients had speech and motor delay, and they had pronounced gait ataxia, intention tremor, and dysidiadochokinesis, findings that are consistent with cerebellar dysfunction; Patients 1-2 and 2-1 were unable to walk. Ataxia was apparent from an early age and was nonprogressive. The results of magnetic resonance imaging (MRI) of the brain, performed in Patients 1-1 and 1-2, were normal (Fig. 1B). Electromyographic studies, performed in three patients, and nerve conduction velocities, assessed in all five patients, were also normal.

Hearing impairment was noted in Patient 1-1 at the age of 5 years (Fig. 1C) and in Patient 2-1 at 1 year. The grade of hearing impairment in Patient 1-1 remained stable over the subsequent 8 years. Patients 1-3 and 1-4 also had sensorineural hearing impairment; Patient 1-2 was not tested.

All affected patients had evidence of stimulated renin systems, with hypokalemic metabolic alkalosis, and they also had hypomagnesemia and hypocalciuria (Table 2).²¹ All patients were receiving potassium and magnesium supplements. Urinary concentrating ability, assessed by means of spot urine osmolality measurements, did not appear to be grossly affected (Table 2). Proteinuria and glycosuria were not present. Ultrasonography showed that the kidneys were normal in size, position, and structure. Blood pressure was at the low end of the normal range, typically at the 25th percentile for age and sex. Other pertinent clinical details are listed in Tables 1 and 2.

LINKAGE ANALYSIS

Family 1 was of Pakistani origin; the affected patients were the offspring of first-cousin marriages who also shared a common ancestor five generations earlier (Fig. 1A). Family 2 was of Arabic descent, and the affected patient's parents were first cousins (Fig. 1A). Whole-genome linkage analysis and a subsequent haplotype reconstruction identified a single region of interest of 1.9 centimorgans (cM), equaling approximately 800,000 bases, with a lod score of 4.98 on chromosome 1 (Fig. 2A and 2B). This region localized between SNPs rs726640 and rs1268524 and contained a total of 31 annotated genes, including *KCNJ10*. Haplotype reconstruction suggested that the affected patients in Family 1 were homozygous by descent for the alleles in the critical region. *KCNJ10*, also known as *Kir4.1*, constituted an attractive candidate gene, since mice in which this gene has been deleted have seizures, ataxia, and sensorineural deafness^{16,22,23}; kidney function in these mice has not been studied. Variations in *KCNJ10* have been reported to be associated with seizure susceptibility in humans,²⁴ although to our knowledge, no mutations in *KCNJ10* have been recorded.

IDENTIFIED MUTATIONS

Sequencing of the complete coding region of *KCNJ10* revealed a homozygous missense mutation, c.194G→C (p.R65P), in the four affected patients in Family 1 and another homozygous missense mutation, c.229G→C (p.G77R), in Patient 2-1 (Fig. 3A and 3B). Parents were heterozygous for the respective mutations (data not shown). Sequencing of 192 ethnically matched control alleles did not reveal the sequence variation p.R65P, identified in Family 1. Likewise, p.G77R, identified in Family 2, was not seen in 108 matched control alleles. Protein-homology analysis revealed that R65 and G77 were conserved among all 21 species studied (Fig. 3C). Residue R65 is predicted to localize to the beginning of the first transmembrane helix of this two-transmembrane-domain potassium channel; G77 also lies within this first transmembrane helix (Fig. 3D).

HETEROLOGOUS EXPRESSION OF *KCNJ10* AND MUTANTS IN *XENOPUS* OOCYTES

Expression of wild-type *KCNJ10* resulted in robust currents with the typical characteristics of an inward-rectifier potassium channel (data not shown). In contrast, currents from mutant R65P *KCNJ10* were reduced to approximately 25% and those from mutant G77R *KCNJ10* to about 5% of wild-type controls (Fig. 3E).

INTRARENAL LOCALIZATION OF *KCNJ10*

KCNJ10 is expressed in the distal tubule of the kidney²⁵; its presence in the thick ascending limb has also been suggested.²⁶ We used a *KCNJ10*-specific antibody in wild-type mice to demonstrate the presence of Kcnj10 distal to the macula densa (i.e., on the basolateral membrane of the distal convoluted tubule, the connecting tubule, and the early cortical collecting duct) (Fig. 4A). The absence of signal in *Kcnj10* knockout mice verified the specificity of the antibody (data not shown).

RENAL PHENOTYPE OF *Kcnj10* KNOCKOUT MICE

Kcnj10 knockout mice die very early as a result of central nervous system symptoms such as seizures.¹⁶ However, a conditional knockout of *Kcnj10* in the brain leads to death later in life, suggesting that renal salt loss is an aggravating factor in the mice with complete knockout.²³ To our knowledge, renal involvement in *Kcnj10* knockout mice has not previously been recognized, although these mice clearly have diminished growth and no weight gain after birth (Fig. 4B).

In our studies, *Kcnj10* knockout mice died after day 8. In the neonatal period, all seven knockout mice had a significantly lower urinary creatinine concentration than did normal mice, indicating polyuria; the urinary sodium concentration was significantly elevated in the knockout mice, indicating renal salt loss. These neonatal knockout mice also had significantly reduced calcium excretion (Fig. 4C), as do patients with the Gitelman syndrome. In addition, the tubulopathy observed in the knockout mice closely resembled that seen in our patients. Urinary findings in 18 heterozygous mice were not significantly different from those in 11 wild-type mice (data not shown).

DISCUSSION

We report a unique constellation of multiorgan signs and symptoms — epilepsy, ataxia, sensorineural deafness, and a renal salt-losing tubulopathy, which we call the EAST syndrome — associated with mutant *KCNJ10*. The multiplicity of symptoms reveals the important role of *KCNJ10* in these various organ systems.

We detected homozygous missense mutations in our patients that substantially impair the function of *KCNJ10*, a potassium-channel gene. The identified mutations reside in a highly conserved area — namely, a transmembrane region that probably determines the overall function of this type of channel.²⁷ Indeed, in a heterologous expression system, these mutations nearly abrogated potassium current. Mice lacking this potassium channel died as neonates; homozygous nonsense mutations or gene deletions of this channel may also be fatal in humans. Alternatively, it is possible that *KCNJ10* has a more important role in mouse kidneys than in human kidneys.²⁸ In any case, our patients are probably in a state of compensated volume, whereas knockout mice are not.

The currently accepted model of renal epithelial salt transport posits that a favorable electrochemical gradient drives the influx of sodium (often transported with other substances) from the tubular lumen into the cell. This gradient is established by the basolateral sodium–potassium pump (Na^+/K^+ -ATPase), which also provides an exit mechanism for sodium. In order for this primary active Na^+/K^+ -ATPase to function, potassium must be able to leave the cell and must be readily available basolaterally.²⁹ The task of *KCNJ10* is to recycle potassium, which is necessary for the function of the primary active Na^+/K^+ -ATPase. This “pump coupling” was postulated in 1958 by Koefoed-Johnsen and Ussing,³⁰ and experimental evidence has been subsequently corroborated by many investigators.^{31,32} Our findings appear to constitute genetic proof for this basic physiological principle. A functional basolateral potassium channel also translates the potassium concentration gradient into a cell-negative transmembrane potential. Since uptake of luminal sodium chloride in the distal convoluted tubule is electroneutral, impairment of the negative membrane voltage itself would not be problematic. However, because the functions of other channels are critically dependent on membrane voltage, impairment of other transport processes (e.g., for chloride or magnesium) can occur.

KCNJ10 activity, according to this view, provides a mechanism for indirectly regulating re-absorption of renal tubular sodium, which modulates volume homeostasis and maintains blood pressure. Indeed, our patients, lacking normal *KCNJ10* activity, had low blood pressure. Moreover, chromosome 1q23.2, where the locus for *KCNJ10* resides, has been implicated repeatedly as being linked to blood-pressure variation in different ethnic groups.^{33–35} In addition, a whole genome scan for the identification of blood-pressure modifiers (as a quantitative trait) in hypertensive and normotensive Lyon rat strains showed linkage to the region syntenic to human chromosome 1q23.2.³⁶

Although the presence of basolateral potassium channels in the renal tubule, including the distal convoluted tubule, has long been known from electrophysiological studies, the molecular identity of these channels has remained unresolved.³⁷ Our results should establish *KCNJ10* as a critical component of basolateral potassium conductance in the distal convoluted tubule. Indeed, similarities in the electrophysiological properties of *KCNJ10* and other known basolateral potassium channels have led to speculation that basolateral potassium conductance is achieved by the *KCNJ10* protein in heteromerization with other potassium-channel proteins.³⁸

Thus, loss of *KCNJ10* function probably leads to a compensated state of salt loss, resulting in stimulation of the renin–angiotensin–aldosterone system and the respective renal tubular activation of potassium secretion in the aldosterone-sensitive nephron (collecting duct). The concomitant proximal tubular increase in bicarbonate (resulting in metabolic alkalosis) and calcium absorption (with consequent hypocalciuria) causes additional signs and symptoms of the EAST syndrome (Tables 1 and 2).

Seizures and ataxia develop in mice with *Kcnj10* deletion, and they die shortly after birth, reflecting the critical role of *KCNJ10* in the functioning of the central nervous system.¹⁶ Even mice with a conditional knockout of *Kcnj10* in glial cells alone die at approximately 3 weeks of age.²³ In humans, *KCNJ10* is expressed in glial cells in the cerebral cortex and cerebellar cortex and in the caudate and putamen, and it is believed to establish the neuronal cell's resting membrane potential through a process called potassium spatial buffering.³⁹ With repeated excitation and repolarization, a neuron takes up substantial amounts of sodium and loses potassium. Thus, potassium accumulates extracellularly, decreasing the membrane potential, facilitating further excitations, and creating a diathesis toward seizures. Glial cells presumably take up the extruded potassium and distribute it through gap junctions; *KCNJ10* has been implicated in this process.¹⁶ We propose that the absence of fully functional *KCNJ10* removes this protective “potassium sink,” accounting for the seizures in our patients.

Investigations in mice have shown that *Kcnj10* is expressed in intermediate cells in the stria vascularis and is required for the generation of the endocochlear potential, suggesting that it contributes indirectly to potassium enrichment of the endolymph.²² This explains why *Kcnj10* knockout mice have markedly impaired hearing. Our patients' hearing was only moderately impaired, and in two patients (1-3 and 1-4), hearing impairment was noted only on specific testing — findings that are consistent with the presence of residual channel function,

Our observations also show further genetic heterogeneity among the salt-losing tubulopathies and establish *KCNJ10* as a basolateral potassium channel that is necessary for proper salt handling in the distal convoluted tubule of the kidney. These observations illustrate the critical role of *KCNJ10* in the human brain and inner ear and provide the basis for further studies of the pathogenesis and potential treatment of epilepsy, movement disorders, and sensorineural deafness. Mutations in the transport genes of the renal tubular lumen often lead to kidney-specific phenotypes, as in the Gitelman syndrome and Bartter's syndrome types I and II.^{2–4,40} Mutations in the basolateral subunit of a renal tubular chloride channel result in clinical findings in the kidney and the ear, as seen in Bartter's syndrome type IV.⁶ We now know that mutations in *KCNJ10* lead to distinct epithelial-transport abnormalities in the kidney and the ear. In other organs and systems, the *KCNJ10* channel plays a major role in modulating resting membrane potentials in excitable cells, causing epilepsy if mutated.

The EAST syndrome should be suspected in patients presenting with any cardinal signs or symptoms of epilepsy, ataxia, or sensorineural deafness, especially if a concurrent electrolyte disorder, such as hypokalemia or hypomagnesemia, is diagnosed.

We speculate that the identification of the genetic basis of the EAST syndrome reveals a key role of *KCNJ10* in the modification of volume homeostasis. Reevaluation of genomewide association studies may identify *KCNJ10* as a candidate gene associated with blood pressure and its regulation.

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Figure 1. Pedigrees of Family 1 and Family 2 and Clinical Findings in Patient 1-1

The pedigree of Family 1 (Panel A) shows four affected members, and that of Family 2 one affected member. Squares indicate male family members and circles female family members; filled squares and circles indicate that the patients are affected. Double lines between parents indicate that the parents are related. An MRI scan of the brain obtained from Patient 1-1 at 9 years of age (Panel B) shows normal cerebral and cerebellar structures. An audiogram of the left ear in the same patient at 14 years of age (Panel C) shows moderate hearing loss, which is more pronounced at higher frequencies. A hearing deficit of up to 20 dB is considered normal for frequencies from 250 to 8000 Hz.

Figure 2. Linkage Studies
A haplotype reconstruction (Panel A) for the locus on chromosome 1 shows identical alleles (indicated by the same color) in the linked region in all affected patients. The numbers (i.e., 1 or 2) next to the alleles indicate the respective status of the single-nucleotide polymorphisms used for genotyping. Recombinations in Patients 1-1 and 1-2 define this region. The parametric multipoint linkage analysis of the whole genome for Family 1 (Panel B) has a single significant peak, with a maximum lod score of almost 5 on chromosome 1. Genetic distance (in centimorgans) and individual chromosomes (1 to 22) are indicated on the lower and upper x axes, respectively.

Figure 3. Sequence Analysis and Functional Studies

Panel A shows sequence chromatograms for a wild-type (WT) control and Patient 1-1. The homozygous missense mutation c.194G C (CGC CCC; p.R65P) is present in the patient's chromatogram (arrow). Panel B shows sequence chromatograms for a wild-type control and Patient 2-1. The homozygous missense mutation c.229 G C (GGC CGC; p.G77R) is present in the patient's chromatogram (arrow). Panel C shows a protein-alignment (homology) plot of the first transmembrane region of *KCNJ10* in 21 vertebrate species, with complete conservation of R65 and G77 (arrows). A model based on the crystal structure of a bacterial homologue of *KCNJ10*, (i.e., *kcsa*), in Panel D, shows the localization of mutations within the first transmembrane region. When heterologous expression of wild-type *KCNJ10* and mutants (R65P and G77R) in xenopus oocytes was measured with the use of a two-electrode voltage clamp, a significant decrease of specific currents in mutant *KCNJ10* was observed, as shown in Panel E. N denotes the number of experiments.

Figure 4. *Kcnj10* in Mice

Serial sections of a kidney from a wild-type mouse (Panel A), stained for antibodies to the proteins NKCC2 (green, indicating luminal thick ascending limb), KCNJ10 (green), NCC (green, indicating luminal distal convoluted tubule), AQP2 (red, indicating principal cells of the luminal collecting duct), and calbindin (blue, indicating distal convoluted tubule and collecting duct) show staining for KCNJ10 basolaterally in the distal part of the nephron only. The glomerulus is indicated by an asterisk. Scale bars indicate 100 μ m. Growth curves for wild-type mice, heterozygous (HE) mice, and homozygous (knockout) mice (based on the mean values for two animals of each type) show virtually no growth of the knockout mice (Panel B), which died after day 8. The photograph of two 6-day-old mice from the same litter, one knockout and one wild-type, shows the growth arrest in the knockout mouse. The bar graph (Panel C) shows the mean results of urinalysis for 7 knockout mice as compared with 29 wild-type and heterozygous mice, all at the age of 3 days. Significant results were obtained for creatinine concentration (Creat) (a decrease), sodium excretion (Na/Creat) (an increase), indicating renal salt wasting, and calcium excretion (Ca/Creat) (a decrease) in the knockout mice.

Table 1

Characteristics of the Patients and Medications.

Variable	Patient 1-1	Patient 1-2	Patient 1-3	Patient 1-4	Patient 2-1
Age at presentation (mo)	5	4	4	4	3
Age at last follow-up (yr)	14.9	9.0	6.4	4.6	3.0
Seizures	Tonic-clonic	Tonic-clonic	Tonic-clonic	Tonic-clonic	Tonic-clonic
Ataxia	Frequent falls	Unable to walk	Frequent falls	Unsteady gait	Not walking yet
Hearing	Sensorineural deafness	Not assessed	Sensorineural deafness	Sensorineural deafness	Sensorineural deafness
Mutations in <i>KCNJ10</i>	p.R65P/p.R65P	p.R65P/p.R65P	p.R65P/p.R65P	p.R65P/p.R65P	p.G77R/p.G77R
Medications and supplements					
Antiepileptic drug	None	Valproate, lamotrigine	Valproate	Valproate	Phenobarbital
Potassium (mmol/kg body weight/day)	8.5	5.2	6.3	7.2	8.2
Magnesium (mmol/kg/day)	0.9	1.2	1.4	1.6	2.0

Table 2

Results of Laboratory Tests.*

Variable	Normal Range	Patient 1-1	Patient 1-2	Patient 1-3	Patient 1-4	Patient 2-1
Plasma						
Sodium (mmol/liter)						
Mean		141	136	139	140	138
Range	133–146	136–143	129–144	137–141	135–145	135–140
Potassium (mmol/liter)						
Mean		3.1	3.2	3.2	3.0	3.2
Range	3.5–5.5	2.6–4.0	2.2–4.3	2.6–4.5	2.5–3.8	3.0–3.8
Chloride (mmol/liter)						
Mean		98	100	99	101	95
Range	100–108	93–101	96–103	96–102	98–104	90–97
Bicarbonate (mmol/liter)						
Mean		30	26	26	27	27
Range	22–26	23–32	15–30	24–30	24–30	23–29
Creatinine (μmol/liter)						
Mean		39	50	45	40	23
Range	20–100	24–46	39–65	36–52	35–47	16–27
Calcium (mmol/liter)						
Mean		2.39	2.42	2.36	2.40	2.47
Range	2.17–2.66	2.17–2.60	2.18–2.68	2.22–2.56	2.29–2.51	2.42–2.54
Magnesium (mmol/liter)						

Variable	Normal Range	Patient 1-1	Patient 1-2	Patient 1-3	Patient 1-4	Patient 2-1
Mean		0.64	0.66	0.57	0.67	0.62
Range	0.66–1.0	0.56–0.77	0.52–0.76	0.47–0.63	0.52–0.78	0.58–0.67
Renin (pmol/ml/hr)						
Mean		15.4	28.6	10.0	7.0	9.9
Range	0.4–3.1					
Urine						
Fractional urinary excretion of sodium (%)						
Mean		0.36	1.51	1.1	0.46	0.8
Range	<1	0.23–0.53	1.23–1.78	0.004–2.2	0.16–0.84	0.7–0.8
Fractional urinary excretion of potassium (%)						
Mean		71	59	54	30	86
Range	5–25	55–112	38–80	43–65	14–60	56–115
Transubular potassium gradient [†]						
Mean		37	24	23	19	26
Range	4–12	28–46			8–29	23–30
Calcium:creatinine ratio (mmol/mmol)						
Mean		<0.07	0.15	0.33	<0.05	0.29
Range	0.2–0.6	<0.01–0.11	0.03–0.16	0.11–0.53	<0.04–0.06	0.2–0.4
Fractional urinary excretion of magnesium (%)						
Mean		5.3	10.4	9.8	4.6	8.1
Range	<4	4.7–6.4	1.24–15.8	4.2–15.4	3.4–6.2	7.6–8.7

Variable	Normal Range	Patient 1-1	Patient 1-2	Patient 1-3	Patient 1-4	Patient 2-1
Osmolality (mOsm/kg)						
Mean		834	573	547	501	724
Range	50–1200	771–894			494–554	694–754
Creatinine (mmol/liter) [‡]						
Mean		4.7	3.0	2.7	3.5	2.1
Range		0.9–8.8	1.5–4.1	1.4–3.6	1.6–6.5	1.7–2.6

^{*} The values for biochemical data were obtained mainly during treatment. To convert the values for creatinine to milligrams per deciliter, divide by 88.4. To convert the values for calcium to milligrams per deciliter, divide by 0.250. To convert the values for magnesium to milliequivalents per deciliter, divide by 0.5.

[‡] The transtubular potassium gradient is an indicator of aldosterone activity.

[‡] No reference range for creatinine is available since creatinine excretion is dependent on fluid intake, muscle mass, and kidney function.