

# Hypokalemic Salt-Losing Tubulopathy With Chronic Renal Failure and Sensorineural Deafness

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**ABSTRACT.** *Objective.* To characterize a rare inherited hypokalemic salt-losing tubulopathy with linkage to chromosome 1p31.

*Methods.* We conducted a retrospective analysis of the clinical data for 7 patients in whom cosegregation of the disease with chromosome 1p31 had been demonstrated. In addition, in 1 kindred, prenatal diagnosis in the second child was established, allowing a prospective clinical evaluation.

*Results.* Clinical presentation of the patients was homogeneous and included premature birth attributable to polyhydramnios, severe renal salt loss, normotensive hyperreninemia, hypokalemic alkalosis, and excessive hyperprostaglandin E-uria, which suggested the diagnosis of hyperprostaglandin E syndrome/antenatal Bartter syndrome. However, the response to indomethacin was only poor, accounting for a more severe variant of the disease. The patients invariably developed chronic renal failure. The majority had extreme growth retardation, and motor development was markedly delayed. In addition, all patients turned out to be deaf.

*Conclusion.* The hypokalemic salt-losing tubulopathy with chronic renal failure and sensorineural deafness represents not only genetically but also clinically a disease entity distinct from hyperprostaglandin E syndrome/antenatal Bartter syndrome. A pleiotropic effect of a single gene defect is most likely causative for syndromic hearing loss. *Pediatrics* 2001;108(1). URL: <http://www.pediatrics.org/cgi/content/full/108/1/e5>; *tubulopathy, Bartter syndrome, polyhydramnios, renal failure, syndromic deafness, pleiotropic gene.*

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ABBREVIATIONS. HPS/aBS, hyperprostaglandin E syndrome/antenatal Bartter syndrome; TAL, thick ascending limb of Henle's loop; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; NKCC2, furosemide-sensitive Na-K-2Cl cotransporter; ROMK, renal outer-medullary potassium channel; SND, sensorineural deafness; GFR, glomerular filtration rate; BERA, brainstem-evoked response audiometry; PPN, partial parenteral nutrition.

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Inherited salt-losing tubulopathies with hypokalemic alkalosis involve an overlapping set of renal tubular disorders that can be subdivided into at least 3 phenotypes: 1) classic Bartter syndrome, 2) Gitelman syndrome, and 3) hyperprostaglandin E syndrome/antenatal Bartter syndrome (HPS/aBS).<sup>1,2</sup> Whereas patients with classic Bartter syndrome and Gitelman syndrome typically present in early infancy and childhood or adolescence, manifestation of HPS/aBS occurs in utero and the neonatal course is severe.<sup>3</sup> The first clinical sign is maternal polyhydramnios caused by fetal polyuria, which regularly results in premature birth between 28 and 34 weeks of gestation.<sup>4,5</sup> Postnatally, affected infants present with the typical pattern of impaired tubular reabsorption in the thick ascending limb of Henle's loop (TAL), including salt wasting, isosthenuric or hyposthenuric polyuria, and hypercalciuria with subsequent medullary nephrocalcinosis.<sup>6-8</sup> Characteristically, endogenous formation of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) is stimulated markedly, resulting in additional aggravation of saluretic polyuria together with fever, vomiting, secretory diarrhea, osteopenia, and failure to thrive.<sup>3</sup> Suppression of enhanced PGE<sub>2</sub> formation with cyclooxygenase inhibitors, such as indomethacin, significantly reduces polyuria and salt wasting and restores normal physical growth.<sup>9,10</sup> Recently, it was demonstrated that prenatal indomethacin treatment could stop additional progression of polyhydramnios, thereby preventing extreme prematurity.<sup>11</sup>

The molecular basis of HPS/aBS is heterogeneous. Mutations in either the furosemide-sensitive Na-K-2Cl cotransporter (NKCC2)<sup>5,12</sup> or the renal outer-medullary potassium channel (ROMK)<sup>4,13</sup> have been found in the majority of HPS/aBS patients. Both proteins are polarized to the apical membrane of the epithelial cells of the TAL, and their physiologic coupling accounts for the reabsorption of 30% of the filtered NaCl load.<sup>14</sup>

In a few cases of HPS/aBS, hearing loss has been reported.<sup>15</sup> This might be related to a high incidence of sensorineural deafness (SND) in preterm infants (up to 10%<sup>16,17</sup>), because HPS/aBS patients are born prematurely. However, Landau et al<sup>18</sup> described an association of "infantile Bartter syndrome" with SND in an inbred Bedouin kindred with at least 5 affected individuals. They proposed that this association may result from the pleiotropic effect of a sin-

gle recessive gene defect. Recently, the disease-causing gene in this family was localized to chromosome 1p31.<sup>19</sup> It remains to be clarified whether a single gene is altered in this inbred family, leading to the tubular disorder as well as SND, or 2 tightly linked genes are responsible for the cosegregation of these phenotypes.

To address the question of whether a third candidate gene might be involved in the cause of HPS/aBS, we previously analyzed the haplotypes of 22 either consanguineous or multiplex families. In 7 kindreds, the haplotypes were suggestive of linkage of the disease to the NKCC2 locus on chromosome 15q21-25 and in 9 kindreds to the ROMK locus on chromosome 11q24. All affected individuals were found to carry mutations in the respective genes.<sup>4,5,20,21</sup> In the remaining 6 families, linkage of the disease to chromosome 1p31 was established recently.<sup>22</sup>

We describe here the phenotype of 8 patients linked to 1p31. In 1 family with a previous index case, the diagnosis in the second child was established by prenatal diagnosis from amniocytes. The postnatal course of this child has been monitored thoroughly.

## METHODS

### Patients

The study cohort comprised 6 kindreds with 8 patients previously diagnosed as having HPS/aBS. All patients were offspring of consanguineous unions. The haplotype data of the 6 kindreds were not compatible with linkage of the disease to the ROMK or the NKCC2 gene locus. Instead, the haplotypes were highly suggestive of linkage to chromosome 1p31. Haplotype data of kindreds II, III, V, and VI were reported recently.<sup>22</sup>

### Prenatal Diagnosis

Genomic DNA was extracted from cultured amniocytes obtained by amniocentesis at 24 weeks of gestation. Linkage of the disease to the 1p31 locus previously found in the elder brother (index case) was demonstrated through analysis of 6 microsatellites linked to 1p31 (D1S2661, D1S417, D1S475, D1S200, D1S2690,

D1S2742). In addition, the chromosomal regions that harbor the genes for NKCC2 and ROMK were excluded as disease-causing loci by haplotype analysis following the protocols previously described.<sup>4,5</sup>

### Clinical Evaluation

Patients were recruited from 5 different pediatric nephrology centers in Germany. The clinical and laboratory findings at first clinical presentation and during follow-up were obtained from hospital records. In 1 infant, whose diagnosis was made prenatally, a prospective evaluation could be initiated immediately after birth. Weight and height standard deviation scores (SDS) were calculated on the basis of growth data from normal Arab children reported by Neyzi et al.<sup>23</sup> Glomerular filtration rate (GFR) was estimated by the creatinine clearance with the use of 24-hour urine collection or of the Schwartz formula.

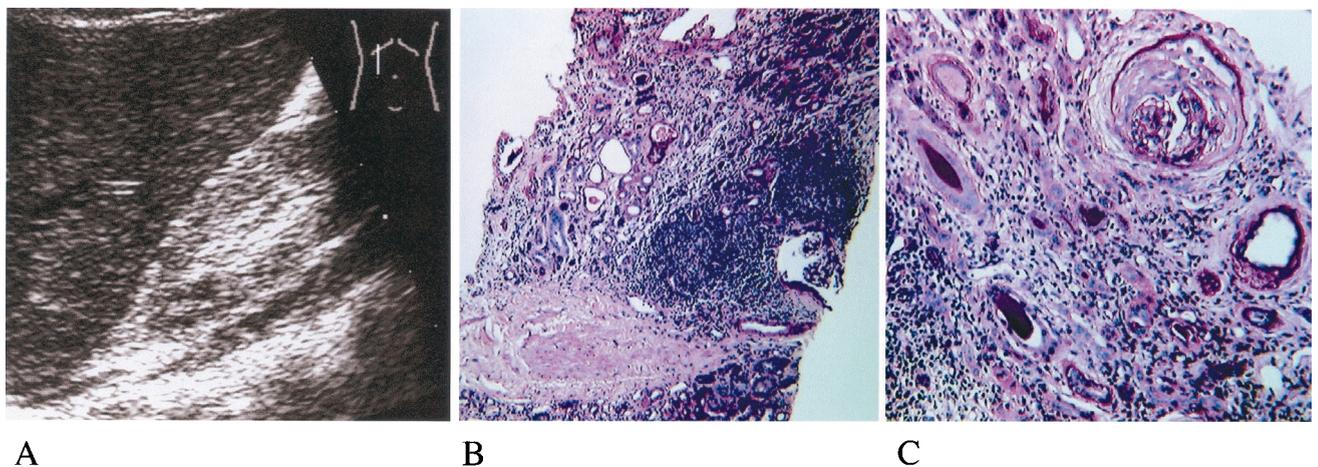
### Laboratory Methods

PGE<sub>2</sub> and PGE-M concentrations were determined by gas chromatography-tandem mass spectrometry as described previously,<sup>24</sup> and the urinary excretion rate corrected for body surface area was calculated. Excretion of PGE-M is regarded as mainly reflecting the extent of systemic PGE<sub>2</sub> formation, whereas urinary PGE<sub>2</sub> represents renal biosynthesis. Plasma level of indomethacin was measured by high-performance liquid chromatography. Active renin and aldosterone were assayed by radioimmunologic methods. The remaining parameters were determined by routine laboratory methods.

## RESULTS

### Case Report of Kindred IV

In the index case of kindred IV (IV-1), prenatal course and postnatal renal salt and water wasting suggested the diagnosis of HPS/aBS. The patient, however, was not treated with indomethacin because of impaired renal function. Beginning at 3 months of age, renal ultrasound showed hyperechoic kidneys (Fig 1A). During the first year of life, urinary calcium excretion was low (0.08 mol/mol creatinine) and glomerular function was persistently impaired with serum creatinine between 1.0 and 1.8 mg/dL. At 1 year of age, complete sensorineural hearing loss was diagnosed. In the second year of life, indomethacin (2 mg/kg/d) and spironolactone (1 mg/kg/d)



**Fig 1.** Morphologic and ultrastructural findings in the kidney of patient IV-1. A, Sonographic view of the right kidney at the age of 20 months showed a hyperechoic parenchyma with loss of corticomedullary differentiation. B, Renal histology: the microscopic overview showed dense mononuclear infiltration throughout the specimen with focal follicle-like clustering (original magnification 160 $\times$ ). C, On higher magnification (400 $\times$ ), predominant tubulointerstitial fibrosis and tubular atrophy involving >40% of the tubulointerstitial compartment were found; furthermore, focal calcifications and dense mononuclear infiltration in the interstitium were found. Twelve of 44 glomeruli were completely sclerosed, whereas some of the remaining ones showed a collapsed capillary convolute and thickening of the basement membrane at the vascular pole of Bowman's capsule.

were added to salt and water replacement. Concomitantly, severe dehydration and electrolyte imbalances, which had resulted in frequent hospital admissions, no longer occurred, although renal salt and fluid losses and plasma renin activity were not completely corrected. A kidney biopsy was performed at 2 years of age. Renal tissue showed marked tubulointerstitial fibrosis and global glomerular sclerosis (Fig 1B and 1C). In addition, typical hypertrophy of the juxtaglomerular apparatus was visible. At the last measured age of 3 years, 9 months, GFR was calculated to be 45 mL/min/1.73 m<sup>2</sup> using the Schwartz formula. Growth was satisfactory with weight and height SDS of -1.2 and -1.0, respectively. Despite decreased muscle tone, the boy has been able to walk alone from 3 years of age on. He is alert and has had a cochlear implant, which further improved the perceptual development. His current medication consists of indomethacin (2.2 mg/kg/d) and spironolactone (0.7 mg/kg/d), in addition to KCl (6 mmol/kg/d) and NaCl (4 mmol/kg/d) supplementation.

During the second pregnancy, polyhydramnios and fetal hydrops with ascites and pleural effusions were diagnosed at 17 weeks of gestation. At 24 weeks of gestation, amniocentesis revealed elevated chloride (114 mmol/L [normal: 108 ± 32<sup>5</sup>]) and aldosterone (170 pg/mL [normal: 110 ± 20<sup>26</sup>]) concentrations in the amniotic fluid. DNA analysis from cultured amniocytes demonstrated linkage to chromosome 1p31, as found previously in the first child (Fig 2). Subsequently, the mother was treated with indomethacin (1.3 mg/kg/d) with careful monitoring of fetal cardiovascular status. Additional progression of polyhydramnios was not observed. At 27 weeks of gestation, digoxin (6.25 µg/kg/d) was added because of progressive fetal effusions. At 30

weeks of gestation, a male infant (IV-2) was born by cesarean section because of fetal distress. Mechanical ventilation was necessary for 20 days. Pleural and abdominal effusions consisted of chyle and were drained for 24 days.

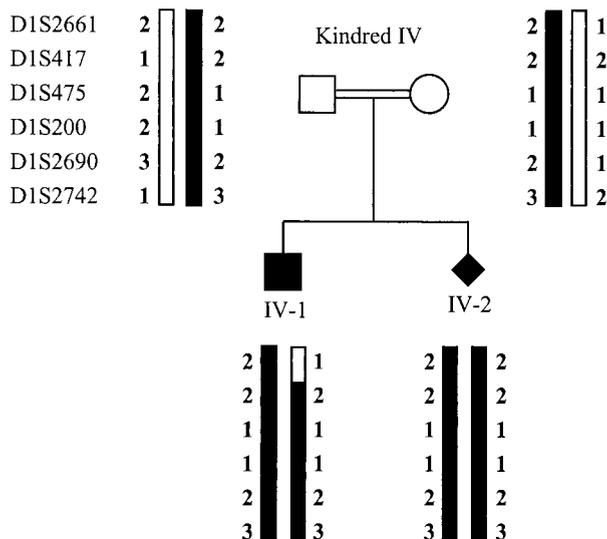
Indomethacin treatment was initiated after an excessive increase in diuresis and urinary chloride and PGE<sub>2</sub> excretion during the first 24 hours of life. Complete suppression of PGE<sub>2</sub> formation by indomethacin resulted in a significant decline of saluretic polyuria but was accompanied by a distinct rise in serum creatinine. During the following weeks, the indomethacin dose was titrated with the aim of decreasing diuresis and saluresis without additional deterioration of glomerular function. Increasing indomethacin doses were required because of the infant's increasing metabolic capacities. Indomethacin plasma concentrations ranged from 42 to 360 ng/mL 4 hours postdosing. When the patient was 10 weeks old and with an indomethacin dose of 2.0 mg/kg/d and an indomethacin plasma concentration at approximately 250 ng/mL, renal PGE<sub>2</sub> formation was normal and serum creatinine was moderately elevated, whereas diuresis and urinary chloride excretion were decreased but not completely normalized (Fig 3).

Within the first 6 weeks, the renal NaCl loss was replaced gradually by the loss of potassium. Urinary potassium excretion in weeks 1, 3, and 6 (periods without indomethacin treatment) was 3, 6, and 10 mmol/kg/d, respectively. Simultaneously noted was a tendency toward lower plasma potassium levels, which could not be influenced by indomethacin treatment.

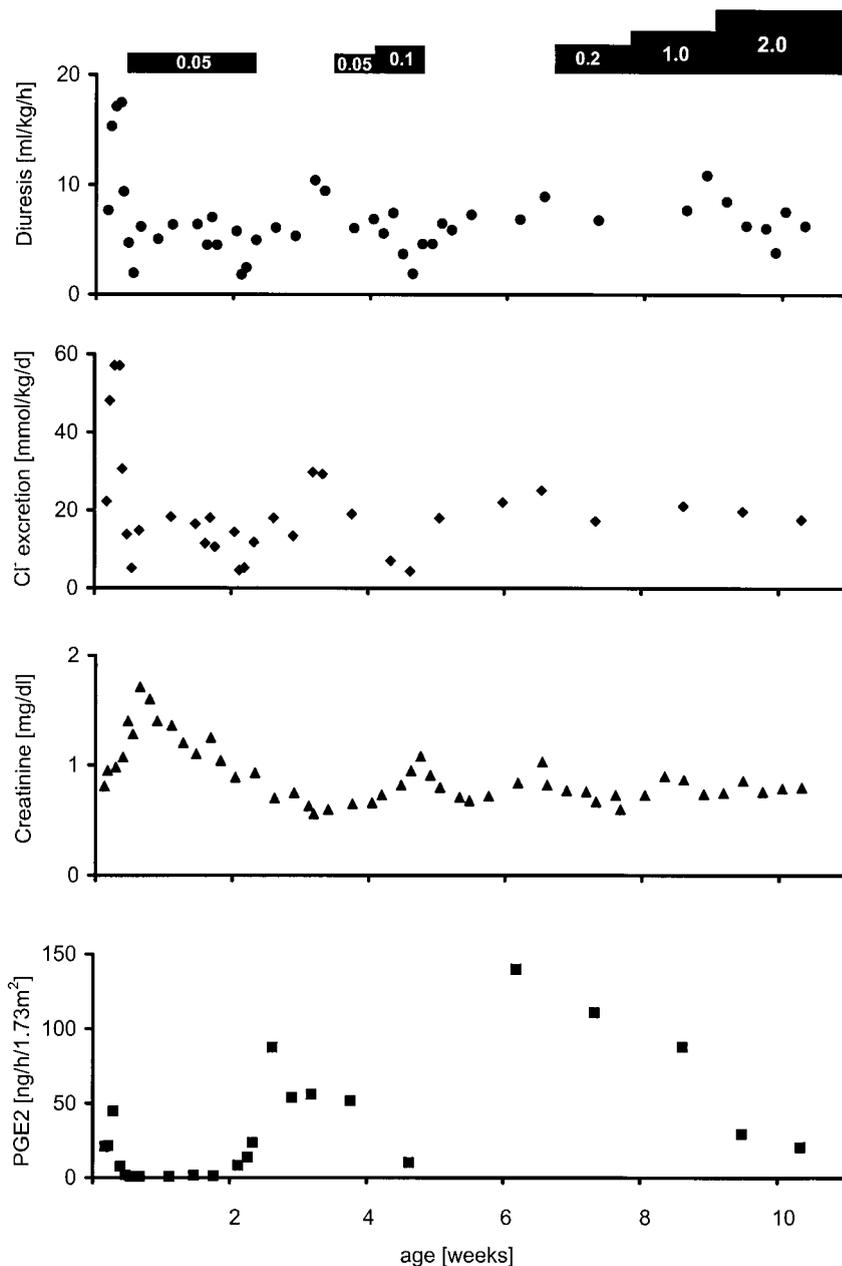
Renal calcium excretion was elevated initially (10 mol/mol creatinine) but decreased to 0.77 mol/mol creatinine at 3 months of age (normal values for preterm infants: 0.57+0.41 mol/mol creatinine<sup>27</sup>). The decline of urinary calcium excretion was independent from indomethacin treatment. Renal ultrasound revealed diffusely echogenic parenchyma at 4 months of age.

Vomiting that was resistant to indomethacin treatment was a major problem in the medical care of the preterm infant and led to introduction of continuous partial parenteral nutrition (PPN). Because vomiting was accompanied by severe metabolic alkalosis (base excess >+10 mmol/L), arginine hydrochloride (1-2 mmol H<sup>+</sup>/kg/d) was added with some beneficial effect. While receiving PPN, growth of the child was satisfactory with weight and height SDS of -1.3 and -1.6, respectively. Discontinuation of PPN from 18 months on resulted in a drop of weight SDS to -2.6 within 6 months.

At the last measured age of 2 years, GFR is calculated to be 37 mL/min/1.73 m<sup>2</sup> using the Schwartz formula. The motor development of the child is delayed markedly. Muscle tone and deep tendon reflexes are decreased generally. The gross motor skills are equal to those of a 6-month-old infant only. In contrast, social behavior has been less affected by the developmental delay despite complete hearing loss, which was confirmed by brainstem-evoked response audiometry (BERA) 3 months after birth. The child



**Fig 2.** Haplotype analysis using DNA from cultured amniocytes demonstrated cosegregation of the disease with the cytogenetic region 1p31 by homozygosity by descent as previously found in the first child. VI-I harbors a maternal recombination nearby (centromeric from) the D1S2661 microsatellite. On the basis of this recombinational event, the critical interval for linkage of the disease recently was refined at its telomeric side.<sup>22</sup>



**Fig 3.** Postnatal follow-up of diuresis, saluresis, plasma creatinine level, and urinary PGE<sub>2</sub> excretion rate. The filled boxes in the upper panel indicate the indomethacin treatment phases with the respective dosage (mg/kg/d).

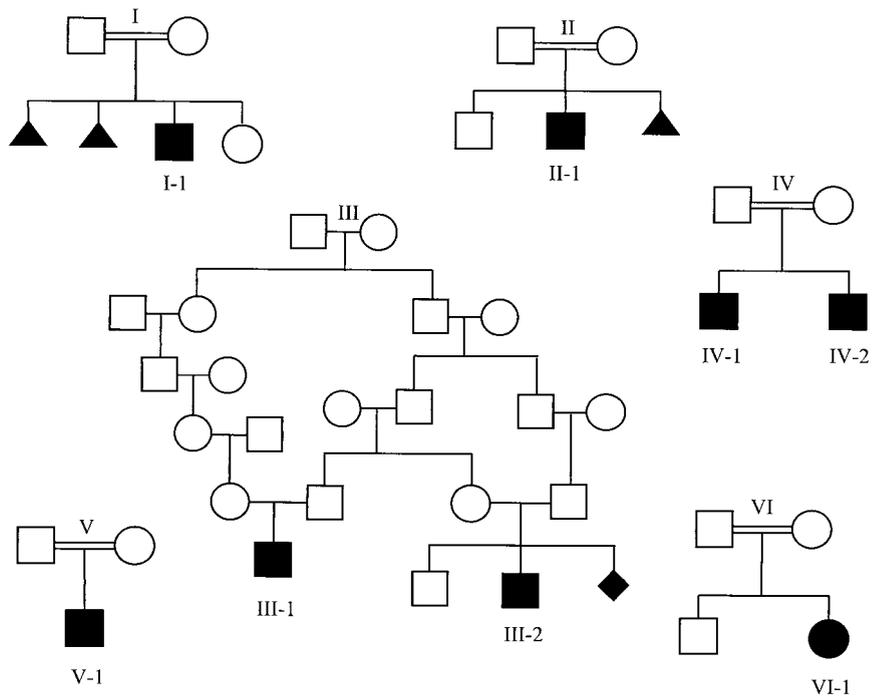
perceives its surroundings attentively and uses non-verbal language—such as eye contact, facial expression, and symbolic gestures—to communicate with others. Current medication consists of indomethacin (3.0 mg/kg/d), KCl (5.3 mmol/kg/d), NaCl (3.8 mmol/kg/d), and arginine hydrochloride (1 mmol H<sup>+</sup>/kg/d).

#### Study Cohort

The pedigrees of the families that originated from either Turkey or Lebanon are depicted in Fig 4. In kindred I, preterm delivery with immediate death of the newborn was recorded twice. In kindred II, 1 therapeutic abortion was induced after diagnosis of fetal hydrops at 21 weeks of gestation. The mother of III-2 is pregnant for the third time. In the third pregnancy, progressive polyhydramnios was observed from 18 weeks of gestation on. Subsequent haplotype

analysis of the cytogenetic region 1p31 revealed concordance between the fetus and the index case III-2 (data not shown).

Important clinical and laboratory findings of the 8 patients are summarized in Table 1. The most prominent symptoms included intrauterine onset, profound renal salt and water wasting, renal failure, SND, and motor retardation. Maternal polyhydramnios was observed at ~20 weeks of gestation. Rapid progression of hydramnios resulted in a median gestational age of only 30 weeks. Duration in the intensive care nursery varied from 3 weeks to 9 months (median: 4 months). During the first 2 years of life, nearly all patients were hospitalized for at least one third of the time. Episodes of vomiting and fever associated with severe volume depletion and electrolyte disturbances were the most common causes for hospital admission.



**Fig 4.** Pedigrees of 6 consanguineous families with linkage of the disease to chromosome 1p31.

**TABLE 1.** Clinical and Biochemical Features of 8 Patients With Linkage to Chromosome 1p31

Feature	I-1	II-1	III-1	III-2	IV-1	IV-2	V-1	VI-1
Origin	Lebanon	Turkey	Turkey	Turkey	Lebanon	Lebanon	Turkey	Turkey
Present age (y)	18	9	3.5†	5	3.75	2	3	6
Age of gestation (wk)	30	31	27	31	30	30	28	33
Birth weight (g)	1430	1710	1130	1350	1500	1930	1120	1590
Diuresis (mL/kg/d)*	307	250	500	300	ND	410	360	200
Chloride excretion (mmol/kg/d)*	30	27	45	36	16	57	36	21
Serum sodium (mmol/L)‡	120	120	130	129	126	128	130	117
Serum chloride (mmol/L)‡	74	70	80	82	76	98	NA	86
Serum potassium (mmol/L)‡	2.7	2.5	2.6	2.6	3.6	2.8	2.7	2.0
Serum bicarbonate (mmol/L)*	36	29.6	NA	32	29.2	31	34.6	NA
Renin*§	NA	78x	36x	>6x	>100x	23x	NA	125x
Aldosterone*§	NA	0.7x	2.8x	2.5x	4.3x	1.6x	NA	5.4x
PGE-uria*								
PGE <sub>2</sub> (ng/h/1.73 m <sup>2</sup> )	NA	238	192	65	84	87	210	27
PGE-M (ng/h/1.73 m <sup>2</sup> )	NA	NA	5370	11635	29026	5090	21800	4880
Maximum urine osmolality (mmol/kg)¶	300	351	320	302	293	359	270	297
Creatinine clearance (mL/min/1.73 m <sup>2</sup> )#	NA	43	16	40	20	28	24	33

\* Maximum values determined within the first month of life.

† Deceased.

‡ Minimum values determined within the first month of life.

§ As the assays for renin and aldosterone differ between the collaborating centers, elevation is given as x-fold above the upper-normal limit.

|| Normal values: PGE<sub>2</sub>, 4–27 ng/h/1.73 m<sup>2</sup>; PGE-M, 110–1140 ng/h/1.73 m<sup>2</sup>.<sup>28</sup>

¶ Random urine samples collected during the total follow-up period (in patients I-1 and VI-1 until end-stage renal disease).

# Determined from 24-hour urine collection at 1 year of age.

### Renal Signs

The basic tubular disorder became evident within the first week of life through profound polyuria and salt wasting. Early laboratory examinations revealed hyponatremia, hypochloremia, metabolic alkalosis, extremely stimulated renin-angiotensin-aldosterone axis, and hyperprostaglandin E-uria (Table 1). Plasma potassium levels <3.0 mmol/L that were associated with hyperkaluria ranging from 10 to 25 mmol/kg/d occurred either simultaneously or with short delay and became next to metabolic alkalosis a

major problem in the therapeutic management. Median potassium requirement at the end of the first year was 10 mmol/kg/d (range: 4–14 mmol/kg/d). Four patients required continual parenteral fluid and electrolyte replacement for the first year of life because of intolerance to the high amounts of supplied oral electrolytes.

The urinary concentration ability was almost completely abolished. Despite states of severe dehydration, the urine osmolality hardly rose above iso-osmolar levels (Table 1). Vasopressin applied to II-1

and V-1 at the age of 1 month and 1 year of life, respectively, failed to increase urine osmolality above 320 mmol/kg. High urinary calcium excretion up to 10 mol calcium/mol creatinine was only transitory and resolved spontaneously within the first months of life. Ultrasound of the kidneys after 6 months consistently revealed diffusely increased echogenicity in both the renal cortex and medulla with loss of definition of corticomedullary differentiation. Typical signs of medullary calcinosis were not detected in any of the patients.

Remarkably, all 8 patients developed chronic renal failure (Fig 5). Early impaired glomerular function first was considered to be related to low renal functional capacity in premature infants, but low GFR persisted with values ranging from 16 to 43 mL/min/1.73 m<sup>2</sup> (median: 28 mL/min/1.73 m<sup>2</sup>) at the end of the first year of life (Table 1). Additional deterioration of renal function was observed in half of the cases. Patients VI-1 and I-1 reached end-stage renal disease at ages 4 and 14 years, respectively, and ultimately underwent renal transplantation, with normal allograft function 2 and 4 years posttransplantation, respectively.

#### Response to Indomethacin Treatment

To suppress cyclooxygenase activity, indomethacin treatment was introduced in 5 of 8 patients within the first months of life. Patients III-1, IV-1, and VI-1 did not receive indomethacin in the neonatal period because of increased plasma creatinine concentration (peak levels 3.0, 2.0, and 1.8 mg/dL, respectively). Discontinuation of indomethacin treatment was necessary in 2 patients (I-1, V-1) because of either hemorrhagic or necrotizing enterocolitis.

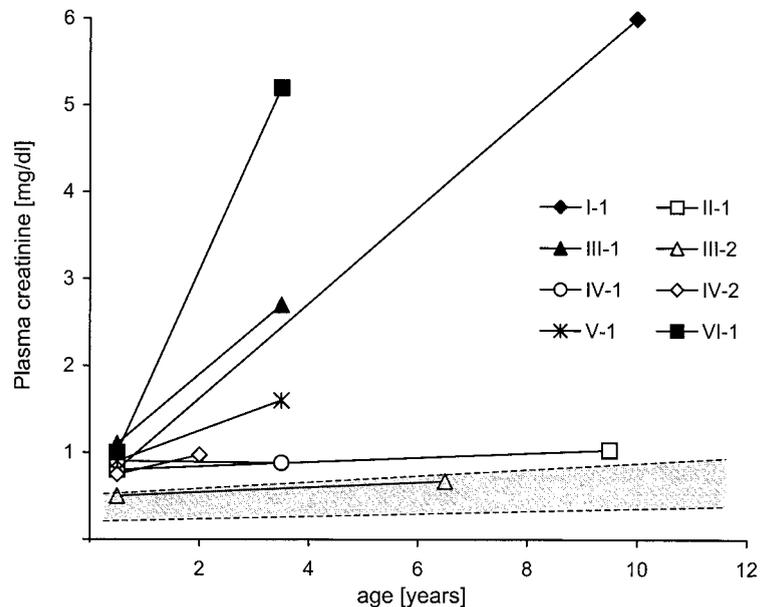
The applied indomethacin doses varied considerably between 0.05 and 9 mg/kg/d, depending on patient age and the response to treatment. In 4 of the 5 early-treated patients, a decline of polyuria and saluresis was observed. However, the beneficial effects in terms of alleviation of symptoms, resolution

of hypokalemic alkalosis, and better growth were far less evident than those formerly described in HPS/aBS patients with mutations in NKCC2 or ROMK.<sup>9–11,29</sup> To maintain normal plasma electrolytes, the patients regularly required additional NaCl and KCl supplies up to 12 mmol/kg/d and 14 mmol/kg/d, respectively. Vomiting and failure to thrive remained major problems in all patients, whether they were on indomethacin or not (Fig 6). At 1 year of age, only 2 patients showed normal physical development in terms of both weight and height. Both patients received PPN. High-calorie diets, via a gastrostomy feeding tube in the majority of patients, had beneficial effects in terms of improving weight but were less effective in accelerating growth.

#### Neurologic Findings

All patients showed severe muscle hypotonia, motor retardation, and complete SND. Major motor milestones were attained with marked delay. Median values for head control and independent sitting were 12 months (range: 9–24 months) and 26 months (range: 18–36 months), respectively. Walking without support was achieved between 3 and 5 years of age. Deep tendon reflexes were normal or only slightly decreased, which may point to a muscular cause of hypotonia. Fine motor skills and coordination were less affected from the motor retardation. Hearing impairment was diagnosed between 3 months and 2 years of age. Subsequent examination by BERA confirmed complete sensorineural hearing loss in all cases. Intellectual skills of the patients were difficult to assess because of deafness, but marked mental retardation was not regularly observed. Patients IV-1 and II-1 received a cochlear implant at ages 3 and 7 years, respectively, with positive effect on speech development. Neonatal cramps occurred in 2 cases; 3 additional patients developed symptomatic convulsions as a result of electrolyte imbalances later in life. However, none of the patients currently require anticonvulsive medication.

**Fig 5.** Follow-up of plasma creatinine levels in 8 patients linked to 1p31. The shadowed area indicates the normal range adjusted for age. Patients who were receiving long-term indomethacin treatment are indicated by open symbols (dose range: 1–3 mg/kg/d). Although patient III-2 remained constantly at the upper-normal limit, this child clearly had impaired glomerular function (Table 1). Severe dystrophia with weight and height SDS between –2 and –4 and, therefore, reduced muscle mass might explain why plasma creatinine concentration did not rise above the upper-normal limit.



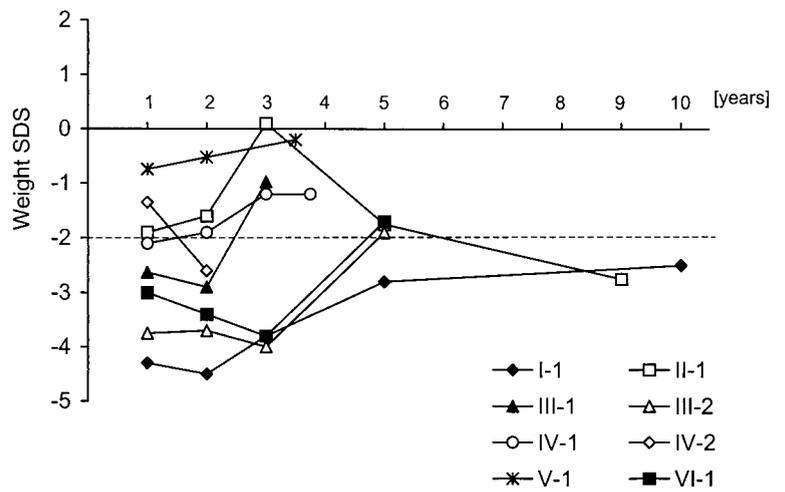
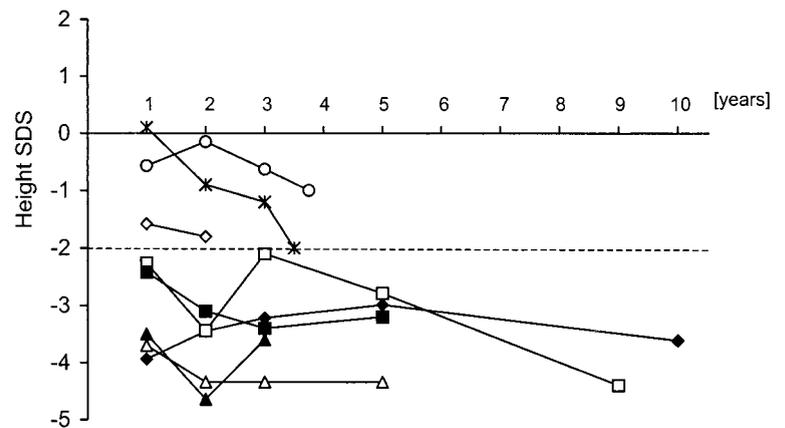


Fig 6. Follow-up of weight and height SDS in the 8 patients. Patients who were receiving long-term indomethacin treatment are indicated by open symbols.



## DISCUSSION

The hypokalemic salt-losing tubulopathy with linkage to chromosome 1p31 seems to be a distinct entity rather than a variant of HPS/aBS. Both diseases share clinical symptoms, such as prenatal onset with polyhydramnios, profound renal salt wasting, impaired urine concentration ability, and failure to thrive. However, the phenotype of our patients is more severe and invariably includes chronic renal failure, SND, and marked motor retardation. The response to indomethacin, the standard therapy in HPS/aBS, is poor, raising the question of the need for a new therapeutic approach.

Different factors may contribute to the more severe manifestation of this disease as compared with HPS/aBS. Our patients revealed a tendency toward lower gestational age at birth than HPS/aBS patients with NKCC2 and ROMK mutations (median: 30 weeks' gestation vs 32 and 33, respectively).<sup>4,5</sup> Lower gestational age might influence renal functional capacity, as it was shown that postnatal development of GFR is slower and urinary sodium excretion is higher in preterm infants with a gestational age of <31 weeks than in preterm infants with a gestational age of 31–34 weeks.<sup>30</sup> In different mammalian species, developmental expression of sodium entry pathways in the distal nephron could be established for NKCC2, ROMK, thiazide-sensitive NaCl cotransporter, and the epithelial sodium channel.<sup>31–33</sup> It is possible that endogenous compensatory mechanisms for alleviat-

ing the primary tubular defect were less functional in our patients. Thus, they were prone to acquire hypovolemic acute renal failure during postnatal adaptation. Prolonged acute renal failure caused by volume contraction may result in ischemic, irreversible injury. This could be aggravated by indomethacin treatment. However, it seems remarkable that renal function tended to deteriorate less in patients who were being treated with moderate doses of indomethacin (Fig 5).

All patients developed chronic renal failure. Potential causative factors include frequent episodes of volume depletion, high doses of indomethacin, chronic hypokalemia and sodium loss, and processes as a result of the inborn disease itself. Normally, severe impairment of glomerular function is a rare complication of hypokalemic salt-losing tubulopathies. Dillon et al<sup>34</sup> described 2 of 10 patients with a GFR below 60 mL/min/1.73 m<sup>2</sup>, and Rudin<sup>35</sup> described only 1 of 28 patients with end-stage renal disease. In our own series of HPS/aBS patients affected by mutations in either NKCC2 ( $N = 12$ ) or ROMK ( $N = 20$ ), only 1 patient had a GFR below 60 mL/min/1.73 m<sup>2</sup>. Chronic indomethacin use seems not to be a risk factor for nonsteroidal antiinflammatory drug-related nephropathy in these patients. In a recent study of HPS/aBS patients who had been treated with indomethacin for >10 years, neither progredient deterioration of renal function nor his-

tologic evidence of indomethacin-related nephropathy was found.<sup>36</sup>

Dillon et al<sup>34</sup> also attributed medullary nephrocalcinosis to impaired glomerular function in HPS/aBS. However, our patients had no signs of medullary calcinosis by ultrasound. In addition, they show only transitory hypercalciuria. This is of note because in HPS/aBS, impaired electrogenic chloride transport in TAL also inhibits voltage-driven, paracellular absorption of calcium, accounting for hypercalciuria. Absence of hypercalciuria could point to a site of the tubular defect other than the TAL. However, an alternative explanation for normal or decreased urinary calcium excretion in our patients might be the decline of GFR, resulting in a lower filtered load of calcium that can be absorbed sufficiently by calcium-transporting pathways other than paracellular absorption in the TAL.

The severe course of the disease certainly was caused by the lack of appropriate treatment. When compared with HPS/aBS, indomethacin had only minor beneficial effects in terms of alleviation of symptoms, decrease of renal salt losses, and partial or complete resolution of hypokalemia. This is remarkable, because PGE<sub>2</sub> formation was extremely elevated in our patients. Besides its participation in renin-angiotensin-aldosterone activation,<sup>37,38</sup> PGE<sub>2</sub> is thought to aggravate renal salt wasting by inhibition of basolateral chloride-channel activity and/or by down-regulation of apical NKCC2 expression in the distal nephron.<sup>39,40</sup> This action most likely is attributable to PGE<sub>2</sub> receptor subtype EP3 receptor-mediated inhibitory effect on cyclic adenosine 3',5' monophosphate production. Cyclooxygenase inhibitors break this vicious circle, thus improving NaCl uptake. The main effect of indomethacin in our patients, however, seemed to be based more on the decrease of GFR and, therefore, reduction of the filtered load rather than on a selective effect on tubular functional capacity.

Increased PGE<sub>2</sub> formation also was found to be related to growth retardation in HPS/aBS by demonstration that indomethacin treatment could prevent failure to thrive and even induce catch-up growth.<sup>3,10,11,34</sup> Our patients, however, did not benefit significantly from indomethacin with respect to better growth. Six of 8 patients required supplementary calories by either gastrostomy feeding tube or long-term PPN to ensure that body weight increased at least to the low-normal limit. Growth was even more impaired in all patients ≥5 years who were far below the low-normal limit for height.

A characteristic sign of the disease in our patients is the constant association with congenital hearing loss. The coincidence of tubular disorder and hearing loss substantiates the previous hypothesis of a pleiotropic effect of a single gene defect.<sup>18,19</sup> Both tubular salt reabsorption and mechano-electrical transduction in the Corti organ rely on the electrochemical gradients across epithelial cell membranes, generated by solute transporters and ion channels. In the past, mutation in several of them were found to account for congenital salt-losing tubulopathies<sup>2</sup> or to cause nonsyndromic or syndromic hearing loss.<sup>41</sup>

It is tempting to speculate whether the candidate gene at chromosome 1p31 encodes a protein that contributes to the transcellular electrolyte transport in both organs, thus indicating a symmetry of renal tubule and inner ear.

Last, an additional neurologic finding, which is different from HPS/aBS, is long-persisting impairment of muscle tone regulation responsible for marked developmental delay. The uniform presentation with muscle hypotonia and posture instability may point to a primary feature of this disease rather than a consequence of prematurity, dystrophia, hypokalemia, and chronic renal failure. However, the cause of motor retardation in our patients needs additional investigation.

Taken together, the phenotype of our patients includes several clinical features that allow for differentiation of this disease from HPS/aBS. It is challenging to diagnose this distinct entity at an early stage, because the standard treatment with indomethacin alone is not sufficient. More effective and complementary therapies still must be established. Apart from correction of renal salt and fluid losses and preservation of glomerular function, medical care must focus on early identification and rehabilitation of hearing loss, motor disability, and growth retardation to maximize the mental, psychosocial, and physical development of these children.

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