

Olivier Devuyst, Hendrica Belge, Martin Konrad, Xavier Jeunemaitre, and Maria-Christina Zennaro

Contents

Barter-Like Syndromes	1202	Familial Hypomagnesemia with Hypercalciuria and Nephrocalcinosis	1228
Introduction	1202	Gitelman Syndrome	1230
Antenatal Bartter Syndrome	1203	EAST/SeSAME Syndrome	1230
Antenatal Bartter Syndrome with Sensorineural Deafness	1209	Isolated Dominant Hypomagnesemia	1230
Classic Bartter Syndrome	1211	Isolated Recessive Hypomagnesemia	1232
Gitelman Syndrome	1215	Hypomagnesemia with Secondary Hypocalcemia	1232
Disorders of the Calcium-Sensing Receptor	1221	Hypomagnesemia with Impaired Brain Development	1234
Familial Hypocalciuric Hypercalcemia, Neonatal Severe Primary Hyperparathyroidism	1222	HNF1B Nephropathy	1235
Neonatal Severe Primary Hyperparathyroidism (NSHPT)	1224	Transient Neonatal Hyperphenylalaninemia	1235
Autosomal Dominant Hypocalcemia	1225	Mitochondrial Hypomagnesemia	1235
Disorders of Magnesium Metabolism	1226	Management of Hypomagnesemia/Magnesium Deficiency	1236
Introduction	1226	Low-Renin Hypertension with Hypokalemia	1236
		Inherited Forms of Primary Aldosteronism	1236
		Pseudohyperaldosteronisms	1239
		Pseudohypoaldosteronisms	1243
		Pseudohypoaldosteronism Type II, Familial Hyperkalemic Hypertension	1243
		Pseudohypoaldosteronism Type I	1246
		References	1252

O. Devuyst (✉) • H. Belge
Zurich Institute for Human Physiology, University of Zurich, Institute of Physiology, Zurich Center for Integrative Human Physiology (ZIHP), Zürich, Switzerland
e-mail: olivier.devuyst@uzh.ch; hendrica.belge@uzh.ch

M. Konrad
Department of General Pediatrics, Pediatric Nephrology, University Hospital, Münster, Germany
e-mail: konradma@uni-muenster.de

X. Jeunemaitre
Department of Molecular Genetics, Hôpital Européen George Pompidou, Paris, France
e-mail: xavier.jeunemaitre@egp.aphp.fr

M.-C. Zennaro
 Inserm U970, Paris Cardiovascular Research Center, Paris, France
e-mail: maria-christina.zennaro@inserm.fr

Bartter-Like Syndromes

Introduction

In 1962, F. Bartter and coworkers described two African American patients presenting a new entity characterized by hypokalemic metabolic alkalosis, renal K^+ wasting, hypertrophy and hyperplasia of the juxtaglomerular apparatus, and normotensive hyperaldosteronism [1]. The disorder also featured increased urinary excretion of prostaglandins, high plasma renin activity, and a resistance to the pressor effects of exogenous angiotensin II [1]. For decades, many similar cases and several phenotypic variants have been progressively identified and included in a group of hypokalemic salt-losing tubulopathies, referred to as Bartter-like syndromes [2]. All these disorders are recessively inherited and associated with hypokalemia and hypochloremic metabolic alkalosis due to stimulation of the renin-angiotensin-aldosterone system (RAAS). However, they differ in terms of age of onset, severity, presence of urinary concentrating defect and/or hypomagnesemia, and magnitude of urinary calcium excretion. Over the years, it became apparent that these tubulopathies affect salt handling in distinct nephron segments, based on the analogy between patient's symptoms and the effects of loop and thiazide diuretics affecting the thick ascending limb (TAL) and the distal convoluted tubule (DCT), respectively.

In the normal nephron, the TAL reabsorbs approximately 25 % of the filtered NaCl load. The apical cotransporter NKCC2 mediates the uptake of Na^+ , K^+ , and Cl^- from the lumen into the epithelial cells, driven by the electrochemical gradient for Na^+ established by the basolateral Na^+K^+ -ATPase. The K^+ channel, ROMK channel, recycles K^+ across the luminal membrane, whereas the Cl^- -Kb channels coupled to their β -subunit barttin are responsible for the basolateral exit of Cl^- (Fig. 1). These transport processes are crucial for the reabsorption of NaCl in the TAL, and thus the urinary concentrating ability, and for generating the lumen-positive electrical charge that drives the paracellular reabsorption of cations (Na^+ , Ca^{2+}

and Mg^{2+}) in this segment [3]. The importance of NKCC2 in the TAL transport is evidenced by the effects of loop diuretics, which, as pharmacologic NKCC2 inhibitors, induce a strong increase in urinary water, salt, and calcium excretion. The DCT is responsible for the reabsorption of 5–10 % of the filtered NaCl [4]. Driven by the activity of the basolateral Na^+K^+ -ATPase, Na^+ enters the DCT cells via the thiazide-sensitive Na^+Cl^- cotransporter, NCCT (or TSC, for thiazide-sensitive cotransporter). Because it is coupled to Na^+ , Cl^- moves into the cell against its electrochemical gradient and then passively exits through the Cl^- -Kb channel in the basolateral membrane. The DCT cells are also involved in K^+ secretion, through the K^+ channel ROMK and a K^+Cl^- cotransporter located in the apical membrane, and the transcellular reabsorption of Ca^{2+} and Mg^{2+} , via the TRPV5/6 and TRPM6 channels, respectively, which belong to the transient receptor potential (TRP) channel family [5]. Thiazide diuretics, which specifically bind and inhibit NCCT, induce a milder diuretic response than loop diuretics, typically associated with magnesium wasting and hypocalcemia.

Based on clinical manifestations, the Bartter-like syndromes were grouped into two major groups: the *antenatal Bartter syndrome* (aBS) (also named hyperprostaglandin-E syndrome (HPS)), which can be associated or not with sensorineural deafness (SND), and the *classic Bartter and Gitelman syndromes* (cBS and GS, respectively). Despite some overlapping features, the aBS group included disorders affecting the TAL, with furosemide-like manifestations, whereas the second group – and GS in particular – was related to a defect in the DCT, with thiazide-like manifestations [2]. From 1996, seminal studies by Lifton and colleagues identified loss-of-function mutations in transporters and channels responsible for these inherited tubulopathies. The aBS was associated to inactivating mutations in the genes coding for NKCC2 [6] and ROMK [7], whereas inactivating mutations in barttin, a regulatory β -subunit of the basolateral Cl^- -Kb channels, were detected in aBS with SND [8]. On the other hand, inactivating mutations of Cl^- -Kb, which is located both in the TAL and DCT, were

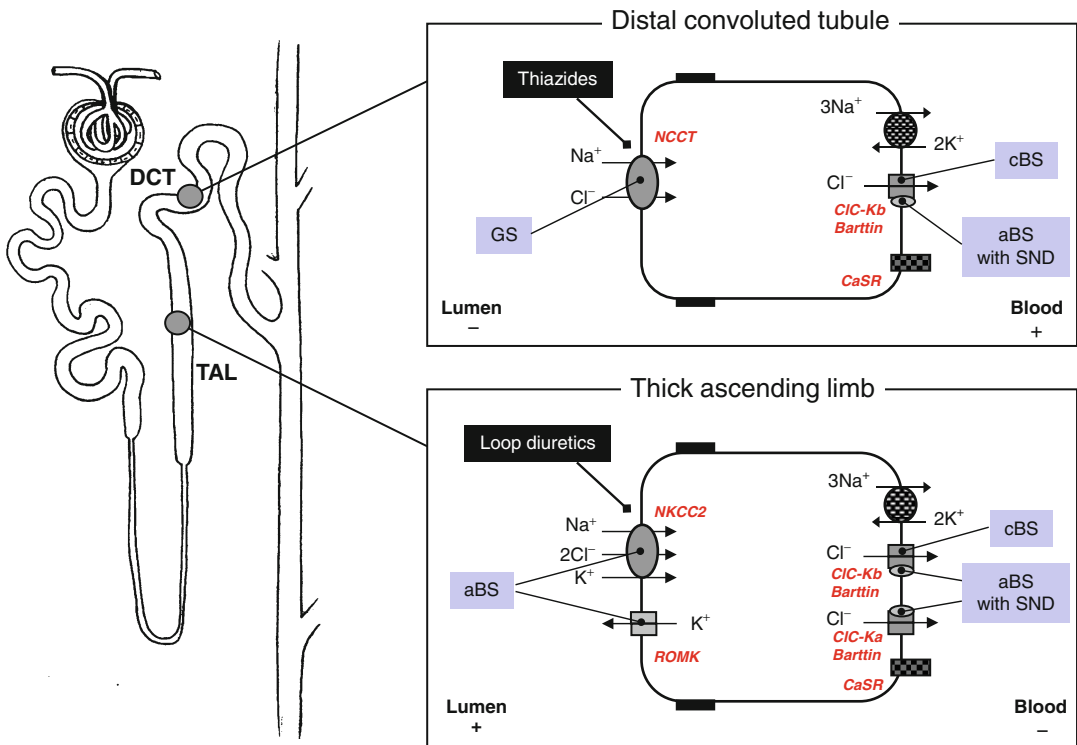


Fig. 1 Molecular basis of Bartter-like syndromes. Approximately 25 % of the filtered NaCl is reabsorbed in the thick ascending limb (TAL) via the apical $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter NKCC2 (inhibited by loop diuretics), organized in parallel with the apical K^+ channel ROMK to ensure K^+ recycling and the lumen-positive voltage. The $\text{Na}^+\text{-K}^+\text{-ATPase}$ and the Cl^- channels CIC-Ka and CIC-Kb associated with the regulatory β -subunit Barttin mediate the exit of Na^+ and Cl^- ions from the cells. The thiazide-sensitive $\text{Na}^+\text{-Cl}^-$ cotransporter NCCT mediates 5–10 % of the NaCl reabsorption in the distal convoluted tubule (DCT). Loss-

of-function mutations in *SLC12A1* (coding for NKCC2) and *KCNJ1* (coding for ROMK) cause antenatal Bartter syndrome (aBS), whereas inactivating mutations of *BSND* encoding the β -subunit barttin cause antenatal Bartter syndrome with sensorineural deafness (aBS with SND) and mutations in *CLCNKB* (CIC-Kb) cause classic Bartter syndrome (cBS). Inactivating mutations in *SLC12A3* (coding for NCCT) are associated with Gitelman syndrome (GS). It must be noted that a few patients with autosomal dominant hypocalcemia due to severe gain-of-function mutations of the CASR may present a salt-losing, Bartter-like tubulopathy.

associated with the cBS [9], whereas GS was found to be associated with mutations of NCCT [10] (Fig. 1).

A classification of these salt-losing tubulopathies, based on the clinical, physiological, and molecular insights discussed above, provides a basis to understand the distinct phenotypes of these disorders (Table 1). When discussing such patients, the clinical diagnosis, based on relatively simple clinical criteria, should be completed whenever possible by the genotyping information since there is only weak genotype–phenotype correlation in these disorders [2, 11].

Antenatal Bartter Syndrome

Genetics

Antenatal Bartter syndrome (aBS, OMIM #601678, #241200) is a rare, life-threatening disorder characterized by massive polyuria that manifests in utero by polyhydramnios and premature delivery in almost all cases. Affected neonates develop salt wasting, hypokalemic metabolic alkalosis, and profound polyuria [12–14].

The disorder is accompanied by markedly elevated urinary PGE_2 excretion, and treatment with PG synthesis inhibitors effectively reduces

Table 1 Inherited Bartter-like salt-losing tubulopathies

Disorder	OMIM #	Inheritance	Gene locus	Gene	Protein	Affected tubular segment
Antenatal Bartter syndrome (aBS), Hyperprostaglandin-E syndrome (HPS), Type I Bartter syndrome ^a	601678	AR	15q15-q21.1	<i>SLC12A1</i>	Na ⁺ -K ⁺ -2Cl ⁻ cotransporter NKCC2	TAL
Antenatal Bartter syndrome (aBS), Hyperprostaglandin-E syndrome (HPS), Type II Bartter syndrome ^a	241200	AR	11q24	<i>KCNJ1</i>	K ⁺ channel ROMK (Kir1.1)	TAL + CCD
Antenatal Bartter syndrome with sensorineural deafness (aBS with SND) ^b , Type IV Bartter syndrome ^a	602522	AR	1p31	<i>BSND</i>	Barttin, beta-subunit of ClC-Ka/b	TAL + DCT
Classic Bartter syndrome (cBS) Type III Bartter syndrome ^a	607364	AR	1p36	<i>CLCNKB</i>	Cl ⁻ channel ClC-Kb	TAL + DCT
Gitelman syndrome (GS)	263800	AR	16q13	<i>SLC12A3</i>	Na ⁺ -Cl ⁻ cotransporter NCCT	DCT

TAL thick ascending limb, CCD cortical collecting duct, DCT distal convoluted tubule

^aThis classification is based on the chronological order of gene discovery

^bA digenic disorder with inactivating mutations of *CLCNKA* and *CLCNKB* has been associated with the aBS with SND phenotype

clinical and biochemical manifestations. The latter features explain why aBS has also been designed as hyperprostaglandin-E syndrome (HPS) [15]. As patients with aBS/HPS fail to respond to loop diuretics, a defective NaCl reabsorption in the TAL was suspected [16]. By combining a candidate gene approach with linkage analysis, Simon et al. demonstrated that aBS/HPS is either due to mutations in NKCC2 (type I BS) or in ROMK (type II BS) [6, 7] (Fig. 1). The two forms of aBS are clinically and biochemically hardly distinguishable [17].

Type I BS is due to mutations in the *SLC12A1* gene located on 15q15-q21.1 containing 26 exons [6, 14, 18]. The *SLC12A1* gene codes for the bumetanide-sensitive NKCC2, a 121 kD protein with 12 putative membrane-spanning domains. NKCC2 is expressed in the apical membrane of epithelial cells lining the TAL and in the macula densa [19]. Loop diuretics bind to portions of transmembrane domains 11 and 12, whereas portions of domains 2, 4, and 7 are involved in ion

transport [20]. Dozens of mutations in *SLC12A1* have been reported, essentially missense or frameshift [21]. The 5 initial kindreds [6] and others reported subsequently [14, 18] were consanguineous, but compound heterozygotes and patients harboring only one heterozygous mutation have been reported [14, 18, 21, 22]. Although a founder mutant allele (W625X) was reported in a cohort of Costa Rican patients [22], the aBS mutations are evenly distributed throughout the *SLC12A1* gene [21]. Of note, alternative splicing of the NKCC2 pre-mRNA results in the formation of three full-length isoforms of NKCC2, which differ in their variable exon 4, their localization along the TAL, and their transport characteristics [23]. Mutations affecting the low-capacity/high-affinity isoform could thus result in a milder phenotype [14].

Type II BS has been linked to mutations in the *KCNJ1* gene that is located on chromosome 11q24 and contains five exons [7]. The *KCNJ1* gene encodes ROMK (also known as Kir1.1), an ATP-sensitive, inwardly rectifying renal

K⁺ channel that is critical for K⁺ recycling in the TAL and K⁺ secretion in the distal nephron [24]. ROMK channels are assembled from four subunits, each consisting of two transmembrane domains flanking a conserved loop that contributes to the pore and selectivity filter and cytoplasmic N and C termini that contain regulatory and oligomerization domains [25]. ROMK exists in three N-terminal splice variations that all behave as rectifying K⁺ channels gated by intracellular pH [24]. Through the recycling of reabsorbed K⁺ back to the lumen, ROMK is believed to be a regulator of NKCC2 cotransporter activity. Therefore, the loss of function in ROMK, as well as in NKCC2, disrupts NaCl reabsorption in the TAL. Mutations in *KCNJ1* that cosegregate with aBS include missense, nonsense, frameshift, and deletions [7, 17, 26–30]. The first mutations were reported in exon 5, common to all ROMK isoforms [7, 17], but homozygous deletions in exons 1 and 2 have also been reported [26, 29].

Clinical Manifestations

Typical features of aBS type I (NKCC2) and type II (ROMK) include polyhydramnios (within the second trimester of gestation); premature delivery (around 32 weeks); severe polyuria; life-threatening episodes of dehydration; hypercalciuria, leading to nephrocalcinosis within the first months of life; and activation of the RAAS (Table 2). The polyuria can be massive (>20 mL/kg/h) despite adequate fluid replacement. Magnesium wasting is not common in aBS [31], although hypomagnesemia was evident in half of the Costa Rican patients [32]. Failure to thrive and growth retardation are invariably observed [13, 32, 33]. A peculiar facies, characterized by a triangularly shaped face, prominent forehead, large eyes, protruding ears, and drooping mouth, has been reported but could reflect dystrophic premature babies [22, 32]. Systemic manifestations including fever of unknown origin, diarrhea, vomiting, and generalized convulsions, which have been attributed to enhanced systemic overproduction of PGE, as well as recurrent urinary tract infection may occur [31, 34]. Osteopenia is common in aBS [13, 35], associated with high urinary excretion of bone resorption

markers [36]. Increased urinary PGE2 excretion is usually detected, although not invariably [2, 13]. Hypophosphatemia with decreased tubular phosphate reabsorption has been described, possibly related to tubular damage and hypokalemic nephropathy [33, 36]. High Cl⁻ and aldosterone concentrations in the amniotic fluid have been reported [13].

Although rare, phenotype variability among NKCC2-deficient patients has been reported, including absence of hypokalemia and/or metabolic alkalosis during the first years of life and persistent metabolic acidosis or hypernatremia [18]. The Costa Rican cohort harboring the W625X founder allele showed a somewhat milder phenotype with a median age of diagnosis at 10 months of life and no necessity of indomethacin treatment in most patients [22, 32]. A late-onset presentation (age 13 and 15 years) with mild polyuria and borderline hypercalciuria has been reported in two brothers harboring compound heterozygous NKCC2 mutations [37].

While renal function is generally well preserved in aBS [18, 32], progressive renal failure leading to ESRD has been reported [32, 38, 39]. The potential mechanisms that could lead to kidney damage in aBS include consequences of early neonatal events and dehydration episodes, hypokalemic nephropathy, nephrocalcinosis, and potential nephrotoxicity of NSAID [38, 40, 41]. Renal biopsies of children with aBS revealed a marked hypertrophy and hyperplasia of the juxtaglomerular apparatus, with stimulation of the renin–angiotensin system [42, 43]. This feature is not specific for aBS and may be observed in all Bartter-like syndromes. Reinalter et al. [41] showed inflammatory infiltrates, with areas of interstitial fibrosis, focal tubular atrophy with thickening of basement membranes and degenerated tubular epithelia, and focal segmental mesangial matrix increase and hypercellularity in renal biopsies obtained in 10 aBS/HPS patients. Of interest, patients harboring ROMK mutations had only minimal histological lesions as compared with NKCC2 patients and cBS patients [41].

Because NKCC2 and ROMK are functionally coupled in the apical membrane of the TAL,

Table 2 Clinical and biochemical features of Bartter-like syndromes

Feature	aBS (<i>SLC12A1</i>) Type I BS	aBS (<i>KCNJ1</i>) Type II BS	aBS with SND (<i>BSND</i>) Type IV BS	cBS (<i>CLCNKB</i>) Type III BS	GS (<i>SLC12A3</i>)
Age of onset	Antenatal	Antenatal	Antenatal	Variable	Childhood, adolescence
Maternal polyhydramnios	Present	Present	Present	Rare	Absent
Prematurity	Present	Present	Present	Rare	Absent
Polyuria	Present	Present	Present	Occasional	Absent
Failure to thrive	Present	Present	Present	Common	Absent
Growth retardation	Present	Present	Present	Common	Occasional
Spasm/tetany/ muscle weakness	Absent	Absent	Absent	Occasional	Present
Nephrocalcinosis	Present	Present	Absent	Occasional, rare	Absent
Sensorineural deafness	Absent	Absent	Present	Absent ^a	Absent
Dehydration episodes	Severe	Severe	Severe		Mild
Hypokalemic metabolic alkalosis	Present	Present (transient neonatal hyperkalemia)	Present	Present	Present
Plasma Mg ²⁺	Normal	Normal	Normal or low	Normal or low	Low
Urinary Ca ²⁺ excretion	High	High	Moderate (transient) or normal	Usually normal	Low
Urinary NaCl excretion	High	High	Very high	Variable increase	Mild increase
Maximal urine osmolality	Hyposthenuria	Hyposthenuria	Iso-/ hyposthenuria	Usually normal	Normal
High renin/ aldosteronism	Present	Present	Present	Present	Present
Urinary PGE2 excretion	High	High	High	Slightly elevated	Usually normal

PGE2 prostaglandin E2

^aSND is present in case of digenic disorder with inactivating mutations of *CLCNKA* and *CLCNKB*

patients with a defective ROMK have a very similar clinical picture than NKCC2 deficiency, with polyhydramnios, premature delivery, severe neonatal polyuria with isosthenuria, and hypercalciuria with secondary nephrocalcinosis [2, 31]. However, there is an important difference in that ROMK-deficient patients show a transient hyperkalemia during the first days of life, correlated with gestational age [31, 44]. The association of such hyperkalemia with hyponatremia and hyperreninemic hyperaldosteronism may

erroneously suggest the diagnosis of pseudohypoaldosteronism type I (PHA1) [44]. Renal K⁺ wasting in these cases may not be apparent until 3–6 weeks postnatally, leading to modest hypokalemia in most patients. Typically, hypokalemia in ROMK-deficient patients is less severe than that observed in NKCC2 patients [29, 31].

By exome sequencing approximately 3000 subjects of the Framingham Heart Study (FHS) cohort, Ji et al. showed that heterozygote carriers of inactivating mutations in NKCC2 and ROMK

had significantly lower systolic and diastolic blood pressure and a significant reduction in the risk of developing hypertension [45]. It was subsequently demonstrated that polymorphisms in *KCNJI* were associated with blood pressure values in a cohort of 2037 adults after adjusting for age, gender, and familial correlations [46]. Since the vast majority of these rare variants in *NKCC2* and *ROMK* have a loss-of-function effect, it can be concluded that such rare variants may exert a profound influence on blood pressure regulation [47, 48].

Differential Diagnosis

Antenatal BS should always be suspected in the face of polyhydramnios due to fetal polyuria. Patients with type II aBS due to *ROMK* deficiency may show a transient hyperkalemia, which can mimic PHA1. However, PHA1 is characterized by permanent hyperkalemia with metabolic acidosis, whereas type II aBS patients typically have metabolic alkalosis, as well as hypercalciuria and nephrocalcinosis. In some patients with aBS, the urinary concentrating defect is so severe that it can lead to hypernatremia, resembling nephrogenic diabetes insipidus [18]. Some patients with aBS may lack metabolic alkalosis during the first year of life or even present a transient metabolic acidosis with defective urinary acidification [49]. This association, which probably results from medullary nephrocalcinosis, can mimic incomplete distal renal tubular acidosis [18]. Other causes of pseudo-Bartter syndromes will be discussed in the section on cBS.

Pathophysiology

Functional investigations of pathogenic *NKCC2* mutations in *Xenopus laevis* oocytes revealed a low expression of normally routed but functionally impaired transporters [50]. Reduced transport function, reduced sodium affinity, and defective processing were also observed when expressing rare *NKCC2* variants associated with low blood pressure in the FHS [51, 52]. A partial intrinsic transport defect was demonstrated in a peculiar mutant (F177Y), which may account for the attenuated phenotype of the affected

patients [37]. Similar functional studies provided insights into the role of *ROMK* in aBS. Two C-terminal mutations located nearby or inside the putative protein kinase A (PKA) phosphorylation site of *ROMK* showed a decreased open probability of the channel [53]. Missense mutations located in the intracellular N and C termini were shown to encode functional channels, but with altered pH gating [28]. Starremans et al. investigated eight *ROMK* mutants and showed that loss of function may result from defective cellular routing to the plasma membrane or impaired channel function [30]. Peters et al. [54] identified defective membrane trafficking in 14/20 naturally occurring *ROMK* mutations and showed that two early in-frame stop mutations could be rescued by aminoglycosides, resulting in full-length *ROMK* and correct trafficking to the plasma membrane. Rare variants of *ROMK* associated with low blood pressure in the FHS also showed reduced *ROMK* channel activity by different mechanisms [55].

The functional data obtained in vitro suggest that loss-of-function mutations in *NKCC2* and *ROMK* disrupt NaCl transport in the TAL, leading to salt wasting, volume contraction, and stimulation of the RAAS. In turn, the distal reabsorption of Na^+ via the epithelial Na^+ channel *ENaC* leads to increased urinary excretion of K^+ and H^+ , causing hypokalemic metabolic alkalosis (Fig. 2). These changes are already observed prenatally, with polyhydramnios, high Cl^- concentrations, and increased aldosterone levels in the amniotic fluid [56, 57]. Chronic volume contraction, elevated levels of angiotensin II, and intracellular Cl^- depletion stimulate PGE2 production, which further inhibits NaCl transport in the TAL and contributes to the washout of the osmotic gradient through enhanced medullary perfusion [2]. The inhibition of *NKCC2* in the macula densa could impair the luminal Cl^- sensing of these cells, which may disrupt the tubuloglomerular feedback and further activate renin release from juxtaglomerular cells [58]. It also appears that cyclooxygenase-2 (COX-2) is induced in the macula densa of children with aBS [59], which could further contribute to hyperreninemia [60]. The early neonatal

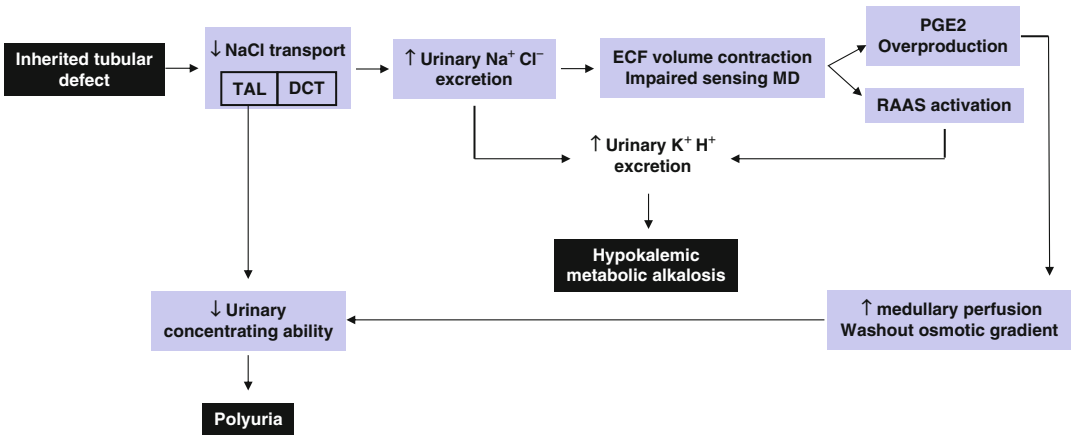


Fig. 2 Physiopathology of hypokalemic metabolic alkalosis and polyuria in Bartter syndrome. See text for details

hyperkalemia harbored by some ROMK-deficient patients is explained by the involvement of ROMK in K^+ secretion in the cortical collecting duct, with subsequent activation of an alternative pathway for K^+ secretion in the cortical collecting duct (CCD) explaining the transient nature of this hyperkalemia [44].

The impaired Cl^- transport in the TAL affects the lumen-positive electrical potential, causing persistent hypercalciuria and early-onset nephrocalcinosis in aBS. Renal Mg^{2+} wasting and overt hypomagnesemia do not reliably segregate with aBS, consistent with the observation that chronic furosemide treatment is not generally associated with hypomagnesemia [61]. Possibly, the loop of Henle or more distal segments may adapt and compensate more efficiently for Mg^{2+} than Ca^{2+} in this syndrome. In the TAL, this adaptation might involve tight junction structures (claudin16/claudin19), whereas increased PGE2 synthesis may contribute to increased Mg^{2+} reabsorption in the DCT [62]. It has been suggested that bone resorption and PGE2-mediated increase in calcitriol may contribute to hypercalciuria in aBS [35, 63]. Schurman et al. [64] showed that elevated levels of angiotensin II may stimulate the release of basic fibroblast growth factor (b-FGF), with a resulting increase in bone resorption via a prostaglandin-dependent mechanism.

NKCC2 knockout (KO) mice show massive perinatal fluid wasting and dehydration, leading to renal failure and death prior to weaning [65]. Treatment of these mice with indomethacin from day 1 allowed 10 % mice to survive until adulthood, despite polyuria, hydronephrosis, hypokalemia, and hypercalciuria. Similarly, the ROMK KO mice manifest early death associated with polyuria, polydipsia, and impaired urinary concentrating ability [66]. Approximately 5 % of these mice survive the perinatal period, but show renal failure, hypernatremia, and metabolic acidosis. Micropuncture analysis revealed that the absorption of NaCl in the TAL was reduced, with severe impairment of the tubuloglomerular feedback. Another strain of ROMK null mouse with a BS-like phenotype showed increased survival to adulthood, due to compensatory mechanisms mostly active in the DCT [67]. Lu et al. demonstrated that the CFTR Cl^- channel may regulate the ATP sensitivity of ROMK in the TAL, which could explain why patients with cystic fibrosis are prone to develop the pseudo-Bartter features of hypokalemic metabolic alkalosis [68].

Treatment

The initial treatment of preterm infants or neonates with aBS should focus on the correction of dehydration and electrolyte disorders, which often

requires continuous saline infusion in a neonatal intensive care unit. Elevated levels of urinary PGE2 provided a rationale for NSAID, and indomethacin has been widely used [2, 13, 31, 33, 49]. Typically, administration of indomethacin starting 4–6 weeks after birth, when massive urinary electrolyte losses have been controlled and hypokalemic metabolic alkalosis is established, corrects both the systemic and biochemical manifestations of aBS [31]. Indomethacin (at doses ranging from 0.5 mg/kg/day to 2.5 mg/kg/day) reduces polyuria, improves hypokalemia, normalizes plasma renin levels, and reduces hypercalciuria [13, 31, 33, 34, 49]. However, the potential benefit of indomethacin administration in premature babies and neonates should be weighed against potential risks of severe gastrointestinal complications, such as ulcers, perforation, and necrotizing enterocolitis [13, 33, 69]. In particular, administration of indomethacin in newborn infants with defective ROMK may be complicated by oliguric renal failure and severe hyperkalemia. At any time, the ROMK-deficient patients are particularly sensitive to indomethacin, with doses well below 1 mg/kg/day sufficient to maintain normal plasma K^+ levels [70]. Similarly, the potential benefit of prenatal treatment with indomethacin [70] should be weighed against the lack of evidence for hyperprostaglandinism in the fetus [13] and the negative effects of NSAID on the ductus arteriosus and the development of the kidney [69]. Selective and nonselective cyclooxygenase inhibitors can also be used for treatment [70]. As an alternative to indomethacin, the COX-2 selective inhibitor, rofecoxib, ameliorated clinical and biological manifestations in aBS patients, with significant suppression of PGE2 and correction of hyperreninemia [60, 71]. However, a case of reversible acute renal failure associated with rofecoxib in an 18-month-old girl with aBS has been reported [72].

Importantly, it should be remembered that HPS is secondary to volume depletion [13] and that the appropriate compensation of fluid and salt wasting remains the essential priority. Additional K^+ supplementation is required, more often for NKCC2-deficient than ROMK-deficient patients [2, 31]. In some cases, a K^+ -sparing diuretic

(usually spironolactone) is necessary to increase serum K^+ levels [13]. Treatment with angiotensin-converting enzyme (ACE) inhibitors has been reported effective in a few cases [33, 73] but should be used with caution as these drugs could block the distal compensatory Na^+ reabsorption. Thiazides should not be used to reduce hypercalciuria, since they interfere with compensatory mechanisms in the DCT and further aggravate dehydration.

The appropriate management of aBS with correction of fluid and electrolyte disorders, indomethacin, and K^+ supplements results in catch-up growth and normal pubertal and intellectual development [13, 33]. However, most patients show a persistent deficiency in height and weight [32, 41]. Correction of hypercalciuria is usually partial, with progression of nephrocalcinosis and a slow decrease in renal function evidenced in some cases [13, 33, 38, 49]. Chaudhuri et al. [39] reported a case of severe aBS in whom preemptive nephrectomy followed by a living-related donor renal transplantation resulted in correction of metabolic abnormalities and excellent graft function. A long-term follow-up study of 15 children with homozygous or compound heterozygous mutations in *SLC12A1* or *KCNJ1* was recently conducted by Puricelli et al. [74]. Under potassium chloride supplementation and indomethacin treatment, these patients presented a satisfactory somatic growth after a median follow-up of more than 10 years. A glomerular filtration rate of <90 mL/min/1.73 m², occasionally associated with overt proteinuria, was present at follow-up in 25 % of the patients, which can be possibly explained by nephrocalcinosis and/or prolonged hypokalemia or by the side effect of the NSAIDs. Of note, 3/15 patients developed gallstones which might represent a new complication of antenatal BS [74].

Antenatal Bartter Syndrome with Sensorineural Deafness

Genetics

In 1995, Landau et al. [75] described a subtype of aBS/HPS associated with sensorineural

deafness (SND) in five affected subjects from an inbred Bedouin kindred. These patients had a severe salt wasting and fluid loss, with poor response to indomethacin and, most often, progressive renal failure [75, 76]. By studying the original kindred, Brennan et al. mapped the disease-causing gene to chromosome 1p31 [77]. In 2001, Birkenhager et al. [8] identified a novel gene, *BSND*, within the critical interval and detected inactivating mutations in affected individuals. This subtype of aBS was named aBS with SND, or type IV BS (OMIM #602522). The original mutations included a splicing mutant, a deletion of two exons, three missense mutations affecting a conserved residue close to the first putative membrane domain, and one mutation resulting in the loss of the start codon [8]. The *BSND* gene consists of four exons. It encodes barttin, a 320-amino-acid protein that contains two putative transmembrane domains and is expressed in the thin limb and thick ascending limb of the loop of Henle and the DCT in the kidney (Fig. 1) and in the stria vascularis surrounding the cochlear duct in the inner ear [8, 78].

Clinical Manifestations

Typically, patients harboring mutations in *BSND* show the most severe form of aBS, with maternal polyhydramnios beginning at week 25 of gestation, severe prematurity, life-threatening neonatal episodes of dehydration, polyuria with hypo- or isosthenuria, and increased urinary PGE₂ excretion [2, 79] (Table 2). The patients are deaf and show a severe growth defect, with delayed motor development [76]. They present multiple episodes of fever, vomiting, and bacterial infections. Jeck et al. [76] reported progressive renal failure in all patients, attributable to glomerular sclerosis and tubular atrophy. However, Shalev et al. reported that early renal failure is not a uniform finding [79]. In contrast with patients harboring mutations in *NKCC2* and *ROMK*, barttin-deficient patients exhibit only moderate and transient hypercalciuria and do not show nephrocalcinosis [2, 79]. This could be due to defective NaCl transport in both the TAL and DCT, with divergent effects on urinary calcium

excretion somehow similar to a combined action of a loop diuretic with a thiazide. Accordingly, barttin-deficient patients may show a severe Mg²⁺ wasting, caused by a defect in both the paracellular (TAL) and transcellular (DCT) pathways of Mg²⁺ reabsorption [2]. Of note, a lack of diuretic response to furosemide and to hydrochlorothiazide was evidenced in one barttin-deficient patient, supporting a defect in both TAL and DCT [80].

There is some degree of phenotype variability among patients with *BSND* mutations. Miyamura et al. [81] reported a patient harboring the loss-of-function G47R mutation of *BSND* who presented at age 28 years with congenital deafness but without polyhydramnios, premature labor, or severe salt wasting in the neonatal period. In contrast, five patients from two unrelated Spanish families harboring the same G47R mutation presented with polyhydramnios, premature birth, and salt loss [82]. Kitanaka et al. [83] reported a patient harboring two mutations in *BSND* (Q32X and G47R) who presented with relatively mild perinatal clinical features but developed end-stage renal failure at age 15 years, requiring renal transplantation. The functional evaluation of the G210S mutation of *BSND* revealed only a very mild disturbance in current–voltage relationship [84], possibly accounting for a milder phenotype [79]. Riazuddin et al. identified a missense mutation (I12T) of *BSND* in four kindreds, associated with nonsyndromic deafness. Of note, the I12T mutation does not affect the chloride channel function and only interferes with the chaperone function of barttin in intracellular trafficking [85]. Functional studies of six *BSND* mutations have revealed that missense mutations can affect the function of ClC-K/barttin despite normal trafficking and membrane insertion of the channels or by binding less efficiently to ClC-K, whereas nonsense mutants interfere with the apical surface sorting [86].

Renal biopsies obtained in patient with *BSND* mutation showed variable features including hyperplasia of the juxtaglomerular apparatus, mild mesangial hypercellularity, mild-to-severe tubulointerstitial fibrosis, areas of tubular atrophy, and sclerosed glomeruli [76, 79].

Pathophysiology

Functional expression studies revealed that barttin is an essential β -subunit for the Cl^- channels CIC-Ka and CIC-Kb, by stimulating Cl^- currents and enhancing surface expression of these channels [78]. Careful functional studies have demonstrated that barttin stimulates chloride flux through the human CIC-Ka by modifying the gating of these double-barreled channels [87]. CIC-Ka and CIC-Kb are two members of the CLC gene family that are located on the basolateral membrane of the cells lining the thin ascending limb (CIC-Ka only), TAL and DCT, and the intercalated cells of the collecting duct. CIC-Ka and CIC-Kb are also expressed in the inner ear, where they colocalize precisely with barttin in specialized, K^+ -secreting cells of the stria vascularis and the vestibular organ [78]. The co-expression of barttin with CIC-Ka/b channels is crucial for NaCl reabsorption in the TAL/DCT (Fig. 1) and K^+ recycling in the inner ear. Disease-causing mutations in barttin disrupt the Cl^- exit from the TAL and DCT, causing the severe salt-losing tubulopathy. Furthermore, the defective barttin/CIC-Ka/b complex impairs the basolateral recycling of Cl^- in the stria vascularis, decreasing the secretion of K^+ into the endolymph and causing SND [78, 88]. The role of barttin as an essential β -subunit for CIC-Ka and CIC-Kb has been substantiated by reports of patients showing a typical aBS with SND phenotype indistinguishable from barttin-deficient patients, but due to a digenic disease caused by loss-of-function mutations in both *CLCNKB* and *CLCNKA* genes [89, 90].

A barttin knockout mouse model was previously generated, but the mice died within days after birth because of severe dehydration [91]. More recently, Nomura et al. generated a knock-in mouse model carrying the disease-causing R8L mutation of *BSND* [92]. These mice presented hypokalemia, metabolic alkalosis, and decreased distal NaCl reabsorption under a low-salt diet. Plasma membrane localization of both mutant barttin and the CIC-K channel was impaired as demonstrated by immunofluorescence and immunoelectron microscopy, and transepithelial chloride transport in the thin

ascending limb of Henle's loop and thiazide-sensitive chloride clearance were significantly reduced. These changes were correlated with the reduced quantity of mutant barttin localized to the plasma membrane [92].

Treatment

Barttin-deficient patients are managed primarily with intravenous fluids in neonatal intensive care units. In contrast with other forms of aBS, and despite high levels of urinary PGE₂, the effect of indomethacin on growth and correction of electrolyte disorders is rather poor [76, 79]. Hypokalemic metabolic alkalosis persists despite high doses of NaCl and KCl supplementation [76]. Zaffanello et al. [80] reported that combined therapy with indomethacin and captopril was needed to discontinue intravenous fluids and improve weight gain in a single patient. A pre-emptive nephrectomy for refractory electrolyte and fluid losses and persistent failure to thrive, followed by peritoneal dialysis and successful renal transplantation, has been reported in a 1-year-old child with type IV BS [39]. Recently, treatment with the Hsp90 inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG), known to rescue ER-trapped mutant proteins in vitro, increased the plasma membrane expression of mutant barttin in the kidney and inner ear and improved the electrolyte disorders and hearing loss of the R8L knock-in mouse model [93].

Classic Bartter Syndrome

Genetics

Classic Bartter syndrome (cBS, or type III BS, OMIM #607364) usually presents during infancy or early childhood, with a phenotype similar to the original description given by Bartter et al. [1], i.e., without the prenatal onset and the nephrocalcinosis seen in the aBS variant. The cBS variant is caused by mutations in the *CLCNKB* gene located on 1p36 [9, 11]. The gene, which contains 19 exons, encodes the basolaterally located renal chloride channel CIC-Kb, which mediates Cl^- efflux from epithelial cells lining the TAL and DCT [9, 88] (Fig. 1).

There is a high rate of deletions encompassing a part of or the entire *CLCNKB* gene [9, 11, 94]. It is hypothesized that the close vicinity of the almost identical *CLCNKA* and *CLCNKB* genes, which are separated by only 11 kb, predisposes to a high rate of rearrangements, for example, by unequal crossing over as demonstrated in two kindreds [9, 11]. In addition, missense, nonsense, small insertion/deletion, frameshift, and splice site mutations have also been reported (<http://www.hgmd.cf.ac.uk>) [95, 96]. A founder mutation (A204T) affecting a highly conserved residue has been reported at the homozygous state in ten patients from nine unrelated, non-consanguineous families in Spain [97]. As expected for a recessively transmitted disorder, a significant number of subjects originate from consanguineous kindred [9, 11].

Pathophysiology

As mentioned earlier (see section on barttin), CIC-Kb is a plasma membrane channel that belongs to the CLC family of chloride channels/exchangers [88]. CIC-Kb and the closely related CIC-Ka isoform are located on the basolateral membrane of the cells lining the thin ascending limb (CIC-Ka only), in TAL and DCT cells, as well as in the intercalated cells of the collecting duct (Fig. 1). They both require the β -subunit barttin to facilitate their insertion in the plasma membrane and generate Cl^- currents [78, 84]. Disease-causing missense mutations of CIC-Kb result in significant reductions or the loss of CIC-Kb/barttin currents [78, 98]. Thus, inactivating mutations in CIC-Kb affect the basolateral exit of Cl^- , which in turn reduces the reabsorption of NaCl in the TAL and DCT. The phenotypic variability of type III BS, which ranges from aBS/HPS in some cases to typical GS in others, may be explained by the wide distribution of CIC-Kb [11, 31] and the fact that alternative pathways for Cl^- exit could partially compensate for CIC-Kb inactivation in the kidney [11, 99]. The nature of *CLCNKB* mutations may also play a role [98, 100]. For instance, functional studies of seven *CLCNKB* mutations in *Xenopus laevis* oocytes revealed two classes of mutants: nonconducting mutants associated with low total protein expression and partially

conducting mutants with unaltered channel properties and CIC-Kb protein abundance [98]. Importantly, none of the type III BS patients with CIC-Kb mutations are deaf, because the function of CIC-Kb/barttin channels in the inner ear can be replaced by CIC-Ka/barttin. Only the disruption of the common β -subunit barttin [78] or the combined loss of CIC-Ka and CIC-Kb [89, 90] results in a Cl^- -recycling defect that lowers K^+ secretion in the stria vascularis to a pathogenic level.

To date, there is no mouse model with targeted deletion of CIC-Kb. Mice lacking CIC-K1 (corresponding to CIC-Ka in humans) show a phenotype of nephrogenic diabetes insipidus, with no modification in the fractional excretion of Na^+ and Cl^- and no hypokalemic alkalosis [101]. These features are caused by the loss of the Cl^- transport across the thin ascending limb, which is essential for generating a hypertonic interstitium [101]. No corresponding human disease linked to loss-of-function mutations of *CLCNKA* has been described. It must be noted that genetic variants of *CLCNKA* have been associated with salt-sensitive hypertension [102]. More recently, a common, loss-of-function variant of *CLCNKA* was identified as a risk factor for Caucasian heart failure [103].

Clinical Manifestations

Patients harboring mutations of CIC-Kb present a broad spectrum of clinical features (Table 2) that range from the aBS/HPS phenotype, with polyhydramnios, isosthenuria, and hypercalciuria, over the classic BS phenotype, with less impaired concentrating ability and normal urinary calcium excretion, to a GS-like phenotype with hypocalciuria and hypomagnesemia [2, 11, 31, 97]. Most patients have episodes of hypokalemic alkalosis and dehydration complicated with muscular hypotonia and lethargy during the first years of life. They are also characterized by increased urinary excretion of PGE2 [2, 11, 31]. The median duration of pregnancy was 38 weeks in the series of Jeck et al. [2], and failure to thrive is common [31, 97]. The diagnosis of Bartter syndrome is usually made during the first year of life, but prenatal (with history of mild maternal polyhydramnios) and late-onset cases are also

reported [104]. Most patients with classic BS show failure to thrive and growth retardation [36, 105, 106]. The latter can be due to profound growth hormone deficiency [107]. Like in the antenatal variants, osteopenia with increased markers of bone resorption can be observed [36].

The electrolyte abnormalities are usually severe at presentation, with low plasma Cl^- and severe hypokalemic alkalosis [95]. Increased plasma renin levels, with high or inappropriately normal (with respect to the hypokalemia) aldosterone levels, are typically observed [11, 31]. Polyuria is not uniformly found in classic BS. Iso-/hyposthenuria was only evidenced in approximately one third of patients, whereas some achieved urinary osmolality above 700 mOsm/kg [2]. The persistence of such a concentrating ability suggests that patients lacking *CIC-Kb* have a residual TAL function. This is further supported by the fact that only ~20 % of patients had sustained hypercalciuria [31]. Nephrocalcinosis was reported in 4/36 affected children [11], but was not detected in three other series [97, 105, 106]. The patients may show a mild hypophosphatemia, which could be related to tubular damage and hypokalemic nephropathy [33, 36]. Isolated cases present with manifestations of renal Fanconi syndrome or distal renal tubular acidosis [97]. About half of the patients lacking *CIC-Kb* have hypomagnesemia [11]. Several patients harboring mutations in *CLCNKB* show overlapping features of cBS (presentation within the first year of life with episodes of dehydration) and GS (hypomagnesemia with hypocalciuria) [11, 99, 108, 109]. Sun et al. [110] reported a patient with cBS who had bilateral sclerochoroidal calcification attributed to persistent hypomagnesemia for 26 years despite magnesium supplementation.

If the full phenotypic spectrum of the Bartter-like syndromes can result from mutations in *CLCNKB*, a significant clinical heterogeneity is observed among patients harboring the same mutation and even between siblings [97, 111]. No correlations between a particular phenotype and *CLCNKB* genotype have been documented yet [11, 99]. It has been suggested that ethnic differences may participate in the phenotype

variability [2]. Indeed, the two original patients described by Bartter were of African Americans origin [1], and early reports suggested that the course of BS may be more severe in African Americans [112]. More recently, Schurman et al. [106] reported significant phenotype variability in the neonatal period in a series of five unrelated African American children with a homozygous deletion of the entire *CLCNKB*.

A vascular hyporeactivity to the infusion of angiotensin II was originally described by Bartter et al. [1]. This feature is not consistently observed, probably because vascular hyporeactivity improves after correction of volume depletion or treatment with NSAID. Extensive studies (reviewed in 113) showed that this vascular hyporeactivity could be due to modifications in the angiotensin II signaling. In turn, these modifications may prevent the release of free radicals – offering increased protection against cardiovascular remodeling in these patients [113]. Stoff et al. [114] evidenced a defect in platelet aggregation in four subjects with Bartter syndrome, but not in other hypokalemic patients. The platelet abnormality was exacerbated by restriction of dietary sodium and lessened by the administration of PG inhibitors. A circulating metabolite of prostacyclin, 6ketoPGE₁, may be responsible for the defect [115].

Bartter syndrome is not classically associated with proteinuria, and renal biopsies consistently show hyperplasia of the juxtaglomerular apparatus, with minimal or no glomerular or tubular abnormalities [1]. However, a few cases of cBS with proteinuria have been reported. Sardani et al. [116] described a 4-year-old African American child with a homozygous deletion in *CLCNKB* and mild mesangial proliferative glomerulonephritis consistent with C1q nephropathy. The follow-up studies of Bettinelli et al. [105] revealed mild-to-moderate glomerular proteinuria in 6/13 *CIC-Kb*-deficient patients. It was associated with decreased GFR in four patients and microhematuria in two. Renal biopsy in two patients revealed diffuse or moderate mesangial hypertrophy [105]. In addition, a few cases of clinical Bartter syndrome with unknown genetic defect presented with focal segmental glomerulosclerosis (FSGS) and renal failure [117, 118]. One

of the patients described by Bartter developed renal failure, with evidence of advanced nephrosclerosis, interstitial fibrosis, tubular atrophy, and glomerular hyalinization [119]. Causes of renal failure in BS include complication of renal salt wasting (including long-standing hypokalemia, hypovolemic episodes, or nephrocalcinosis), chronic activation of the RAAS with ensuing stimulation of TGF-beta and/or TNF-alpha, and toxicity of NSAID [38, 116, 118]. Renal dysfunction was temporally associated with NSAID therapy in two cBS patients, with biopsy-proven interstitial nephritis and resolution after NSAID withdrawal [38]. However, the pathogenic role of NSAID in causing renal damage in Bartter syndrome has been questioned by the nature and topology of the histological lesions, the fact that renal lesions were identified in some patients before initiation of AINS, and the lack of progression of tubulointerstitial lesions over more than a decade under NSAID treatment [41, 120]. Of note, renal cysts have been identified in patients with classic BS [33, 121], potentially linked to renal K^+ wasting and secondary aldosteronism [122].

Jeck et al. identified the common T481S variant in *CLCNKB*, which showed significantly increased currents when expressed in oocytes [123] and was associated with essential hypertension in a German cohort [124]. The relevance of these findings has been discussed [125], and linkage of the T481S variant to high blood pressure was not confirmed in a Japanese cohort [126].

Differential Diagnosis, Unusual Associations, Pseudo-Bartter Syndromes

The differential diagnosis of cBS includes the surreptitious use of loop diuretics and laxative abuse [127], which are both unusual in children [128], and chronic vomiting [129]. Measurement of urinary Cl^- and urine screen for diuretics are usually useful to diagnose these patients [130, 131].

The association of hypokalemic metabolic alkalosis with hyperreninemic secondary aldosteronism is also found in other familial disorders affecting the kidneys or the gastrointestinal tract or can be acquired. Generalized dysfunction of the

proximal tubule (renal Fanconi syndrome), for instance, due to cystinosis [132], or Kearns–Sayre syndrome, a mitochondrial cytopathy caused by large deletions in mitochondrial DNA leading to cytochrome c oxidase deficiency [133], can be associated with biochemical features resembling BS. A case of familial renal dysplasia with hypokalemic alkalosis has been reported [134]. Patients with cystic fibrosis are prone to develop episodes of hyponatremic, hypochloremic dehydration with metabolic alkalosis [135, 136]. As mentioned earlier, the Cl^- channel CFTR, which is mutated in cystic fibrosis, may regulate the function of ROMK in the TAL [68]. Gastrointestinal malformations which are associated with Cl^- deficiency [137], or Hirschsprung disease [138], can also lead to pseudo-Bartter syndrome. Administration of prostaglandins in neonates with a ductus-dependent congenital cardiopathy [139], aminoglycosides [140, 141], or combined chemotherapy [142] can also induce the biochemical features of BS. Bartter syndrome has also been reported in association with autoimmune diseases, for instance, with Sjogren syndrome [143, 144]. Güllner et al. [145] described a syndrome of familial hypokalemic alkalosis in a sibship presenting with hyperreninemia, aldosteronism, high urinary prostaglandin E2 excretion, normal BP, and resistance to angiotensin II. At variance with BS, the patients had hypouricemia, indicative of proximal tubule (PT) dysfunction, and the fractional chloride reabsorption in the TAL was normal. The renal biopsy showed an extreme hypertrophy of the PT basement membranes, whereas the juxtaglomerular apparatus was of normal appearance. The molecular basis of this familial tubulopathy remains unknown. Finally, the association of BS with a partially empty sella detected by MRI of the brain has been reported in both adult and pediatric patients [146, 147].

Treatment

Patients with cBS are typically treated with PG synthetase inhibitors and escalating doses of KCl, complemented with K^+ -sparing diuretics (most often spironolactone) and NaCl in some of them [41, 105, 106]. Indomethacin is the most frequently used drug, usually started within the first

4 years of life at doses ranging from 1 to 2.5 mg/kg/day. Doses above 3 mg/kg/day are considered nephrotoxic. Indomethacin is well tolerated, but one should remain cautious for signs of gastrointestinal complications [33] or alteration of renal function [38, 105]. Selective COX2 inhibitors, such as rofecoxib, have been used instead of indomethacin [33] and are currently evaluated on a larger scale. Potassium supplementation (usually KCl, 1–3 mmol/kg/day) is mandatory in cBS, as hypokalemia is often severe at presentation and is not fully corrected by indomethacin [106]. If KCl alone fails to correct hypokalemia, then addition of spironolactone (1–1.5 mg/kg/day) is recommended. The use of ACE inhibitors, which have been used for treating hypokalemia in adults with BS [148], should be with caution given the risk of hypotension. Magnesium supplementation should be added when hypomagnesemia is present, but the correction is typically difficult [31]. Some patients with cBS require gastrostomy tube placement and enteral feeding [106].

The long-term efficacy of the standard treatment with indomethacin and KCl supplementation has been established in cBS. Most biochemical features improve with therapy, although K^+ levels are typically difficult to normalize in most patients despite NSAID, KCl, and spironolactone. Treatment also results in improved height and weight, but catch-up growth is inadequate and there is persistent height retardation [33, 41, 105, 106]. Recently, growth hormone deficiency has been demonstrated in some patients, with a positive effect of recombinant human hormone treatment [105, 107]. As discussed above, some cases of cBS are complicated by chronic renal failure. A few cases of living-related kidney transplantation have been reported, with improvement of biochemical and hormonal abnormalities after transplantation [117, 120, 149].

Perioperative management of patients with cBS requires a particular care for volume repletion and correction of electrolyte abnormalities during anesthesia and the continuation of antiprostaglandin therapy to prevent the defective platelet aggregation [150, 151].

Gitelman Syndrome

Genetics

Gitelman syndrome (GS) (OMIM #263800) is generally considered as a milder disorder than BS and, with a prevalence of ~1 per 40,000, arguably the most frequent inherited tubulopathy detected in adults [40]. The syndrome was first described in 1966 by Gitelman and coworkers as a familial disorder in which patients presented with hypokalemic alkalosis and a peculiar susceptibility to carpopedal spasm and tetany due to hypomagnesemia [152]. For more than 20 years, GS was assimilated with BS. In 1992, Bettinelli and coworkers concluded that GS could be distinguished from BS, based on low urinary Ca^{2+} excretion (molar urinary calcium/creatinine ratio less than or equal to 0.20) [153]. Also, BS patients were more often born after pregnancies complicated by polyhydramnios or premature delivery and had short stature, polyuria, polydipsia, and tendency to dehydration during infancy and childhood, whereas GS patients presented tetanic episodes or short stature at school age [153]. The dissociation of renal Ca^{2+} and Mg^{2+} handling in GS, together with the subnormal response of these patients to thiazides [154, 155], pointed to a primary defect in the DCT.

In 1996, Simon and colleagues demonstrated that presumable loss-of-function mutations in the *SLC12A3* gene were responsible for GS [10]. *SLC12A3* is located on chromosome 16q13 and comprises 26 exons. It encodes the thiazide-sensitive Na^+-Cl^- cotransporter (NCCT), a 1021-amino-acid integral membrane protein expressed in the apical membrane of cells lining the DCT (Fig. 1). NCCT belongs to the family of electroneutral cation–chloride-coupled cotransporters (*SLC12*) that also includes the $Na^+-K^+-2Cl^-$ and the K^+-Cl^- cotransporters [156]. NCCT contains a central hydrophobic region comprising 12 putative transmembrane domains flanked by a short N-terminal and a long C-terminal hydrophilic intracellular terminus [157]. A model suggests that the affinity-modifying residues for Cl^- are located within TM 1–7 and for thiazides between TM 8 and 12 and that both segments are implicated in defining Na^+ affinity of NCCT [158].

Gitelman syndrome is transmitted as an autosomal recessive trait, and the majority of patients are compound heterozygous for different mutations in *SLC12A3*. To date, more than 180 mutations [96, 159] scattered through *SLC12A3* have been identified in GS patients. Most (~75 %) are missense mutations substituting conserved amino acid residues, whereas nonsense, frameshift, and splice site defects and gene rearrangements are less frequent. A significant number of GS patients, up to 40 % in some series, are found to carry only a single mutation in *SLC12A3*, instead of being compound heterozygous or homozygous [96, 160–162]. Because GS is recessively inherited, it is likely that there is a failure to identify the second mutation in regulatory fragments, 5' or 3' untranslated regions, or deeper intronic sequences of *SLC12A3* or that there are large genomic rearrangements. Nozu et al. were the first to report a deep intronic *SLC12A3* mutation in a GS patient, creating a new donor splice site that results in the inclusion of a novel cryptic exon in mRNA [163]. Lo et al. confirmed that deep intronic mutations in *SLC12A3* causing defective expression of NCCT can be detected with an RNA-based approach in GS patients [164]. They suggested that the analysis of cDNA from blood leukocytes to detect deep intronic mutations should be considered for GS patients who have undetectable or uniallelic *SLC12A3* mutations [164]. Vargas-Poussou et al. showed that there is a predisposition to large rearrangements caused by the presence of repeated sequences within the *SLC12A3* gene. These large rearrangements, which may account for up to 6 % of all *SLC12A3* mutations, can be detected by multiplex ligation-dependent probe amplification (MLPA), enhancing the sensitivity of genetic testing to >80 % [96]. As discussed above, mutations in *CLCNKB* have been detected in a few patients presenting simultaneous features of cBS and GS [99, 108, 109]. The distribution of CIC-Kb in both the TAL and DCT, and potential compensation by other Cl^- transporters, may probably explain these overlapping syndromes [2]. In any case, GS is indeed genetically heterogeneous, raising the possibility of a concurrent heterozygous mutation in a gene other than

SLC12A3. In addition to *CLCNKB*, other genes participating in the complex handling of Na^+ , Ca^{2+} , and Mg^{2+} in DCT, or its regulation, are potential candidates [165].

Pathophysiology

The functional effects of mutant NCCT were tested using *Xenopus laevis* oocytes [162, 166–168]. Functional analyses revealed that some mutant NCCT proteins were synthesized but not properly glycosylated, targeted for degradation, and not delivered to the plasma membrane [166]. Another class of *SLC12A3* mutations results in normal glycosylated proteins partly impaired in their routing and insertion that perform normal function once they reach the plasma membrane [167, 168]. Some mutant NCCTs affect the intrinsic activity of the cotransporter, with normal glycosylation and plasma membrane insertion [162]. Finally, splicing mutations of *SLC12A3* result in truncated transcripts that trigger nonsense-mediated decay (NMD), an mRNA surveillance pathway that allows cells to degrade mRNA that contains premature translation stop codons [162]. Taken together, these results imply that GS may arise from impaired protein synthesis (splicing mutants), defective processing, defective protein insertion of functional mutants, and defective intrinsic activity of the mutant NCCT in the DCT cells [162].

Schultheis and colleagues generated a mouse model with a null mutation in the *Slc12a3* gene on a mixed background [169]. The NCCT null mice showed hypocalciuria and hypomagnesemia at baseline but, in marked contrast to GS patients, no hypokalemic metabolic alkalosis. The NCCT-deficient mice had no signs of hypovolemia on a standard Na^+ diet, but they showed a lower blood pressure than those of wild type when fed a Na^+ -depleted diet for 2 weeks suggesting a subtle hypovolemia compensated at baseline. Subsequent studies performed on a homogeneous C57BL/6 strain showed that NCCT null mice had a mild compensated alkalosis with increased levels of plasma aldosterone [170] and an increased sensitivity to develop hypokalemia when exposed to dietary K^+ reduction [171]. Belge and coworkers showed that mice lacking parvalbumin, a cytosolic Ca^{2+} -binding protein

that is selectively expressed in the DCT, had a phenotype resembling GS, with volume contraction, aldosteronism and renal K^+ loss at baseline, impaired response to hydrochlorothiazide, and higher bone mineral density [172]. They demonstrated that these modifications were due to modifications in intracellular Ca^{2+} signaling and decreased expression of NCCT in the DCT [172].

Studies of inactivating *SLC12A3* mutations and mouse models indicate that the GS phenotype results from dysfunction of NCCT. The loss of NCCT in the DCT leads to salt wasting, volume contraction, stimulation of the RAAS, and increased excretion of K^+ and H^+ in the collecting duct resulting in hypokalemic metabolic alkalosis. By contrast, the pathogenesis of hypocalciuria and hypomagnesemia remains debated. Two hypotheses prevail respecting hypocalciuria. First, the volume contraction causes a compensatory increase in proximal Na^+ reabsorption, driving passive Ca^{2+} transport in the PT [173]. Second, the epithelial cells of the DCT hyperpolarize, due to lower intracellular Cl^- activity, which opens the apical voltage-dependent Ca^{2+} channels (TRPV5), resulting in increased Ca^{2+} influx and reabsorption [174]. Such hyperpolarization could also stimulate the basolateral Na^+/Ca^{2+} exchanger, further increasing Ca^{2+} reabsorption [175]. Studies performed in chronic hydrochlorothiazide-treated mice [176] favor the first hypothesis, as micropuncture experiments demonstrated increased reabsorption of Na^+ and Ca^{2+} in the proximal tubule, whereas Ca^{2+} reabsorption in the distal convolution was unaffected. Furthermore, micropuncture experiments performed in NCCT-deficient mice revealed an enhanced fractional reabsorption of Na^+ and Ca^{2+} upstream of the DCT to compensate the transport defect in that segment [170]. As suggested recently, the state of the extracellular fluid volume – substantially reduced or not – may exert a key role in the type of tubular mechanism involved [177].

Hypomagnesemia is an essential feature of GS. Several mechanisms, including K^+ depletion, increased passive Mg^{2+} secretion, or defective active Mg^{2+} transport in the DCT, have been proposed to explain the Mg^{2+} wasting in GS

[4, 178]. The identification of TRPM6 as a Mg^{2+} -permeable channel in the DCT and its involvement in the pathogenesis of autosomal recessive hypomagnesemia (*see below*) suggested that this channel constitutes the apical entry step in active renal Mg^{2+} reabsorption [179]. Indeed, chronic thiazide administration increased Mg^{2+} excretion and reduced renal expression levels of TRPM6 in mice. In addition, TRPM6 expression was also drastically decreased in mice lacking NCCT [176]. These results suggest that the pathogenesis of hypomagnesemia in chronic thiazide treatment as well as GS could involve TRPM6 downregulation. Structural variations in the epithelial cells lining DCT, with decreased absorptive surface area for Mg^{2+} , may also play a role [169, 180].

A large body of evidence has emerged that a complex signaling cascade involving a set of kinases (with-no-lysine kinases, WNK; sterile 20/SPS1-related proline/alanine-rich kinase, SPAK; oxidative stress-responsive kinase-1, OSR1) can activate members of the SCL12A family by phosphorylation of conserved Ser/Thr residues in their N-terminal domain. The importance of this pathway has been confirmed by the manifestations of GS caused by defective NCCT and pNCCT expression, observed in the SPAK null mice generated by Yang et al. [181].

Clinical Manifestations

Classically, GS was considered as a benign variant of Bartter-like syndromes, usually detected during adolescence or adulthood. Since the disorder originates from the DCT, the salt and water losses in GS patients are less pronounced than in aBS or cBS because urinary concentrating ability should not be affected (Table 2). The GS patients are often asymptomatic or presenting with mild symptoms such as weakness, fatigue, salt craving, thirst, nocturia, constipation, or cramps. They may also consult for growth retardation and short stature, reflecting an alteration in the growth hormone–insulin-like growth factor I axis or pleiotropic effects resulting from magnesium depletion [182]. Typical manifestations include muscle weakness, carpopedal spasms, or tetanic episodes triggered by hypomagnesemia

Table 3 Clinical manifestations associated with Gitelman syndrome

Most common (>50 % of patients)	Prominent (20–50 % of patients)	Occasional (Less than 20 %)	Rare (Case reports)
Salt craving Cramps, muscle weakness, pain Fatigue Dizziness Nocturia Thirst, polydipsia Paresthesia, numbness Palpitations Low blood pressure	Fainting Polyuria Arthralgia Chondrocalcinosis Prolonged corrected QT interval Febrile episodes	Early onset (before age 6) Failure to thrive Growth retardation Vertigo, ataxia Carpopedal spasm, tetany Vomiting Constipation Enuresis Paralysis	Seizure Ventricular tachycardia Rhabdomyolysis Blurred vision Pseudotumor cerebri Sclerochoroidal calcifications

[61, 183]. Blood pressure is reduced, particularly for patients with severe hypokalemia and hypomagnesemia [184]. However, high blood pressure should not rule out the diagnosis, particularly in older patients. Indeed, mild hypertension has been repeatedly reported in GS patients [185, 186]. Since Mg^{2+} ions increase the solubility of calcium pyrophosphate crystals and are important for the activity of pyrophosphatases, hypomagnesemia may promote the formation of calcium pyrophosphate crystals in joints and sclera, leading to chondrocalcinosis [187] and sclerochoroidal calcifications [188]. Patients with GS have higher bone mineral density, similar to chronic thiazide treatment, which likely arises from increased renal Ca^{2+} reabsorption and a decreased rate of bone remodeling [189]. Potassium and Mg^{2+} depletion prolong the duration of the action potential in cardiomyocytes, resulting in prolonged QT interval in ~ 50 % of the patients, which could lead to an increased risk for ventricular arrhythmias [190, 191]. Cases of GS patient who presented with long runs of ventricular tachycardia [192] or ventricular fibrillation with favorable outcome after cardioversion and continuous supplementation [193] have been reported. In addition, hypokalemic rhabdomyolysis has been reported in several (BS and) GS patients [194–196]. Pregnancies in GS appear to have a favorable outcome, provided continuous K^+ and Mg^{2+} supplementation and monitoring for oligohydramnios [197]. A summary of the manifestations associated with GS and their frequency is shown in Table 3.

The classical biochemical features of GS include hypokalemic metabolic alkalosis, hypomagnesemia, and hypocalciuria. The presence of both hypomagnesemia (<0.75 mM) and hypocalciuria (molar urinary calcium/creatinine ratio <0.2) is highly predictive for the diagnosis of GS [153]. The criteria for hypocalciuria in infants or children with GS have been precise [198]. However, there are inter- and intraindividual variations in the extent of hypocalciuria, and hypomagnesemia may be absent in some patients [2, 31, 199]. In addition, the combination of hypocalciuria and hypomagnesemia is also detected in rare cases of cBS patients [108]. Although the urinary PGE_2 excretion is classically normal in GS, increased values can also be detected in some patients with GS [2]. Recently, Vigano et al. showed a hitherto unrecognized tendency toward renal phosphate wasting with mild-to-moderate hypophosphatemia that is not related to an altered circulating level of either 25-hydroxyvitamin D or parathyroid hormone [200]. Several studies described abnormal glucose metabolism and insulin secretion, as well as an increased risk for the development of type 2 diabetes in GS patients, probably due to hypokalemia and hypomagnesemia [201–203].

No specific findings are observed at renal biopsy, apart from occasional hypertrophy of the juxtaglomerular apparatus and markedly reduced expression of NCCT by immunohistochemistry [204]. Interestingly, a few cases of GS associated with glomerular abnormalities and/or proteinuria

have been described. Focal segmental glomerulosclerosis was described in a 21-year-old patient who underwent a renal biopsy for evaluation of chronic hypokalemia [205]. Hanevold et al. [206] reported a case of focal segmental glomerulosclerosis and C1q nephropathy in an African American child with GS who subsequently developed nephrotic range proteinuria. Another case of focal segmental glomerulosclerosis was reported by Ceri et al. in a 32-year-old man with GS and persistent proteinuria [207]. A retrospective analysis of GS patients revealed proteinuria with preserved renal function in 6/36 patients. Three of these six patients presented hypertension. No renal biopsy was performed [186]. Recently, Demoulin et al. reported the first case of genetically proven GS with glomerular proteinuria and significant abnormalities of the glomerular basement membrane (GBM) [208]. Interestingly, defects of the GBM and podocytes were also detected in the *Slc12a3* knockout mouse model. These observations point to the possible link between the functional loss of NCCT and podocyte dysfunction. Possible mechanisms include angiotensin II- or renin-induced podocyte lesions, as well as chronic hypokalemia [208].

Phenotype Variability and Potential Severity of GS

The view that GS is a benign condition has been challenged by reports emphasizing the phenotype variability and the potential severity of the disease. A detailed evaluation of 50 adult GS patients with identified *SLC12A3* mutations revealed that GS was associated with a significant reduction in the quality of life – similar to that associated with congestive heart failure or diabetes [209]. Manifestations such as early onset (before age 6 years), growth retardation, invalidating chondrocalcinosis, tetany, rhabdomyolysis, seizures, and ventricular arrhythmia have been described, although in a limited number of cases [31, 162, 182, 192, 209]. Based on the large number of patients harboring *SLC12A3* mutations, the phenotype of GS is highly heterogeneous in terms of age at presentation, biochemical abnormalities, and clinical manifestations (Table 3). The phenotype variability has

been documented not only between patients carrying different *SLC12A3* mutations but also for a common underlying mutation [210] and between affected family members [162, 211].

The mechanisms that could account for intrafamilial variability include gender (affected brothers are apparently more severely affected than their sisters carrying the same mutation), modifier genes (affecting the regulation or activity of NCCT), and environmental factors (dietary intake of NaCl, Ca²⁺, or Mg²⁺) [2, 162, 211, 212]. Compensatory mechanisms operating in other nephron segments should also be considered, as evidenced in mice with defective NCCT [170, 172, 176]. Finally, considering that most of patients with GS are compound heterozygous harboring various mutant *SLC12A3* alleles, the phenotype variability could be related to the nature and/or position of the underlying mutation(s). This hypothesis has been substantiated by the studies of Riveira-Munoz et al. [162] which showed that a specific combination of mutations was preferentially associated with a severe presentation of GS.

Recently Balavoine et al. showed a more severe phenotype in GS patients with two mutated alleles than in those with one or no mutated alleles [185]. A follow-up study in a large cohort of 117 Taiwanese GS patients demonstrated that male patients had an earlier age of onset, more severe hypokalemia, and significantly lower serum aldosterone concentration and that patients with homozygous and deep mutations in intron 13 had a more severe phenotype [202]. Conversely, Berry et al. reported an apparently more severe electrolyte disturbance in women, which could possibly be due to the effects of female hormones on expression or function of NCCT [186].

Blood Pressure in GS: Effect of the Carrier State

In 2001, Cruz et al. investigated a large Amish kindred to show that patients with GS had significantly lower age- and gender-adjusted diastolic and systolic blood pressure, a higher urinary Na⁺ excretion, and a higher salt intake than their wild-type relatives [184]. Additional support for the

role of NCCT in blood pressure regulation was provided by the report that transplantation of a GS kidney into a non-Gitelman hypertensive recipient resulted in the correction of hypertension in the latter [213]. Considering that the frequency of heterozygote carriers of *SLC12A3* mutations is approximately 1 %, the question was thus raised whether single loss-of-function mutations in *SLC12A3* may affect blood pressure regulation in the general population [47]. Lifton and colleagues screened 3125 adult subjects from the Framingham Heart Study for mutations in *SLC12A3* (and by extension *SLC12A1* and *KCNJ1*, responsible for aBS) and identified 30 different mutations (15 in *SLC12A3*, 10 in *SLC12A1*, and 5 in *KCNJ1*) in 49 subjects [45]. Of these mutations, 10 were biochemically proven loss of function (7 in NCCT alone) and 20 were inferred from the conservation and rarity criteria. Examination of long-term BP revealed that 80 % of the mutation carriers were below the mean systolic BP values of the entire cohort. The mean BP reduction in carriers was similar to values obtained with chronic thiazide treatment [45]. Thus, rare functional variants of three genes involved in Bartter-like syndromes, including GS, have a significant impact in the heritability of BP variation.

Differential Diagnosis

The differential diagnosis of GS includes other Bartter-like syndromes (Table 1), particularly cBS due to mutations in *CLCNKB* [2, 199] as well as diuretic or laxative abuse, and chronic vomiting. As mentioned above, the clinical history and biochemical features, even hypocalciuria and hypomagnesemia, may not be fully reliable to distinguish GS from cBS. Although implementation of genetic testing should be promoted, such testing in the context of the BS and GS bears a significant cost, considering the number of exons to be screened, the lack of hot spots, and the large number of mutations described. Colussi et al. evaluated the response to a simple thiazide test in the diagnosis of GS [199]. They monitored the chloride fractional clearance during the 3 h following the administration of hydrochlorothiazide (HCTZ, 1 mg/kg or 50 mg in adults) orally.

More than 90 % (38/41) of patients with GS showed a blunted response (<2.3 %) to HCTZ, a feature that was never observed in seven patients with BS (five with aBS and two with cBS) and three patients with diuretic abuse or vomiting. Thus, the HCTZ test offers a high sensitivity and specificity for the diagnosis of GS [199]. However, it should not be recommended to diagnose aBS, in view of the specific clinical history and the potential danger of diuretic treatment in these patients. Whether this test has the power to distinguish between the overlapping features of GS and cBS due to *CLCNKB* mutations is also uncertain [214].

Gitelman syndrome-like manifestations including hypokalemic metabolic alkalosis with hypomagnesemia and hypocalciuria have been reported as a rare complication of the use of cisplatin [215]. Although the mechanism remains uncertain, cisplatin is known to induce focal tubular necrosis lesions in the DCT [216]. Autoimmune disorders cause acquired renal tubular disorders, potentially due to autoantibodies against tubular components [217]. Typical features of acquired GS have been reported in association with various autoimmune disorders including iritis and arthritis [218], sialadenitis [144], and Sjögren syndrome [219]. Of note, there was no improvement of renal K^+ wasting after corticosteroid treatment in one such case [219].

Treatment

Lifelong oral magnesium and potassium supplementations are the main treatment in patients with GS. Magnesium supplementation should be considered first, since Mg^{2+} repletion will facilitate K^+ repletion and reduce the risk of tetany and other complications related to hypomagnesemia [183, 220]. All types of magnesium salts are effective, but their bioavailability is variable. Magnesium chloride, magnesium lactate, and magnesium aspartate show higher bioavailability [183]. $MgCl_2$ is recommended since it will also correct the urinary loss of Cl^- . The dose of magnesium must be adjusted individually in three to four daily administrations, with diarrhea being the limiting factor. In addition to magnesium, high doses of oral KCl supplements (up to 10 mg/kg/day in children) may be

required [221]. Importantly, Mg^{2+} and K^+ supplementation results in a catch-up growth [162, 182]. Spironolactone or amiloride can be useful, both to increase serum K^+ levels in patients resistant to KCl supplements and to treat Mg^{2+} depletion that is worsened by elevated aldosterone levels [222]. Both drugs should be started cautiously to avoid hypotension. Patients should not be refrained from their usual salt craving, particularly if they practice a regular physical activity. More recently, the potassium-sparing diuretic and aldosterone antagonist eplerenone was shown to be useful in the treatment of GS patients [223–226]. Eplerenone is a selective aldosterone antagonist, with significantly lower affinity for androgen, progesterone, and glucocorticoid receptors in comparison with spironolactone, and has therefore no antiandrogenic side effects (such as gynecomastia, hirsutism, erectile dysfunction, and menstrual irregularities) [223]. The usefulness of eplerenone was initially demonstrated in patients with cardiovascular disease and heart failure [227]. Prostaglandin inhibitors such as indomethacin are less indicated in GS than in aBS, since urinary PGE_2 levels are usually normal. Liaw et al. reported an improvement in growth response following high-dose indomethacin, but complicated by gastrointestinal hemorrhage [228]. Refractory hypokalemia has also been treated with the specific COX-2 inhibitor rofecoxib [229]. Considering the occurrence of prolonged QT interval in up to half GS patients [190, 191], QT-prolonging medications should be used with caution. Recently, an open-label, randomized, crossover study was conducted to compare the efficacy and safety of 6-week treatment with one time daily 75 mg slow-release indomethacin, 150 mg eplerenone, or 20 mg amiloride added to constant potassium and magnesium supplementation in 30 GS patients [226]. Each drug increased plasma potassium concentration. Indomethacin was the most effective, but it was associated with decreased eGFR and it can cause gastrointestinal intolerance. Amiloride and eplerenone had similar but lower efficacies and increased sodium depletion [226].

Although GS adversely affects the quality of life [184], we lack information about the long-

term outcome of these patients. Renal function and growth appear to be normal, provided lifelong supplementation [230]. Progression to renal failure is rare in GS: only two patients with GS who developed end-stage renal disease have been reported [231, 232]. However, in the follow-up study of a large cohort of 117 Taiwanese patients, 7/117 (6 %) patients developed chronic kidney disease (stages III–IV) and 5/117 (4.3 %) patients developed type 2 diabetes at follow-up (mean duration, 6.7 ± 3.4 years; range, 1–33 years) [202]. In addition, despite the salt wasting, the development of secondary hypertension may be expected in the aging GS population [186].

Disorders of the Calcium-Sensing Receptor

The extracellular Ca^{2+} -sensing receptor (CaSR) is a G protein-coupled receptor belonging to the metabotropic glutamate receptor subfamily that was identified in 1993 by Brown, Hebert, and colleagues [233]. The human *CASR* gene is located on chromosome 3q21 with a coding region of 3234 bp and six exons [234]. The CaSR is ~120 kDa protein forming homodimers through interactions of cysteine residues in the extracellular domain [235]. The CaSR is predominantly expressed in the apical membrane of the parathyroid hormone (PTH)-secreting cells in the parathyroids, in the kidney, in the apical membrane of PT cells and principal cells of the medullary CD, and on the basolateral membrane of cells lining the TAL and DCT [236]. The CaSR regulates the PTH secretion and modulates the renal tubular reabsorption of Ca^{2+} and Mg^{2+} in response to ionized serum Ca^{2+} and Mg^{2+} concentrations [236, 237].

The CaSR responds to physiologically relevant, millimolar concentrations of extracellular Ca^{2+} [Ca^{2+}_o], with a half-maximal response (EC50) of 3 mM. It also shows a distinct affinity for various multivalent cations in vitro, including Mg^{2+} (EC50, 10 mM) [233]. Activation of the CaSR mediates different, cell-specific signal transduction pathways [236]. In bovine parathyroid cells, high levels of [Ca^{2+}_o] activate phospholipase C (PLC) via a member of the Gq family, followed by

the breakdown of phosphatidylinositol 4,5-bisphosphate with formation of 1,2-sn-diacylglycerol and of inositol 1,4,5-trisphosphate (IP3). The accumulation of IP3 leads to the release of intracellular pools of Ca^{2+} causing inhibition of PTH secretion through mechanisms that remain to be fully defined [236]. Microperfusion studies in rat revealed that elevation of peritubular $[\text{Ca}^{2+}_o]$ and $[\text{Mg}^{2+}_o]$ markedly reduces the fractional absorption of Ca^{2+} , Mg^{2+} , and Na^+ in the TAL [238]. As discussed above (see section on BS), the reabsorption of Ca^{2+} and Mg^{2+} in the TAL occurs mainly through a paracellular pathway driven by a lumen-positive, transepithelial potential generated by the combined activity of NKCC2 and ROMK (Fig. 1). High $[\text{Ca}^{2+}_o]$ in the TAL decreases hormone-dependent cAMP accumulation, reflecting a direct inhibition of the CaSR-dependent G alpha-adenylate cyclase (AC) activity [236]. In turn, the reduced cAMP levels decrease NaCl transport, hence Ca^{2+} and Mg^{2+} reabsorption. In addition, Ca^{2+} -induced activation of the CaSR leads to production of arachidonic acid and its metabolites which inhibit the activity of ROMK and NKCC2 activity, further enhancing the reduction of Ca^{2+} and Mg^{2+} transport [239].

Additional evidence of the role of the CaSR in regulating tubular reabsorption of Ca^{2+} and Mg^{2+} was provided by the identification of different types of mutations in the *CASR* gene [240]. Loss-of-function CaSR mutations result in familial hypocalciuric hypercalcemia (FHH) and neonatal severe primary hyperparathyroidism (NSHPT) [241, 242], whereas gain-of-function CaSR mutations result in autosomal dominant hypocalcemia (ADH) [243, 244], which can be associated with a Bartter-like syndrome [245, 246] (Table 4; Fig. 3). The prevalence of FHH is up to one in 16,000 and ADH one in 70,000, whereas NSHPT is very rare [247].

Familial Hypocalciuric Hypercalcemia, Neonatal Severe Primary Hyperparathyroidism

In 1972, Foley et al. described familial hypocalciuric hypercalcemia (FHH), also named

familial benign hypercalcemia (FBH), (OMIM#145980), an autosomal dominant disorder characterized by a mild-to-moderate hypercalcemia, with mild hypermagnesemia, inappropriately normal or mildly elevated serum PTH levels, and hypocalciuria [248]. Although patients with FHH are usually asymptomatic, complications such as chondrocalcinosis, acute pancreatitis, and gallstones may occur with age [249, 250]. A simple diagnostic test is a calcium over creatinine clearance ratio (CCCR) <0.01 [251]. The finding of hypercalcemia in first-degree relatives supports the diagnosis, particularly when found in children under age 10 years.

Calcium infusion studies in FHH patients revealed a higher-than-usual set point for the release of PTH, suggesting an alteration in Ca^{2+} sensing [252]. Genetic linkage studies mapped the gene for FHH to the region of chromosome 3 where the CaSR gene was located, and mutational analyses of the *CASR* gene revealed unique heterozygous mutations in approximately 90 % of the FHH kindreds examined [240, 253, 254]. Isolated cases of FHH with a de novo mutation in *CASR* have also been reported [255, 256]. Many *CASR* mutations cluster in aspartate- and glutamate-rich regions of the extracellular domain of the receptor, which may act as cationic binding sites [240]. Expression studies confirmed that FHH-causing mutations induce a rightward shift of the set point for the Ca^{2+} -dependent responses, corresponding to a loss of function [242, 244]. The defective extracellular CaSR likely leads to inappropriate absorption of Ca^{2+} and Mg^{2+} in the TAL [239] and Mg^{2+} transport in the DCT [237]. Renal excretion of Ca^{2+} and Mg^{2+} is reduced, which leads to hypercalcemia and sometimes hypermagnesemia [257].

FHH is genetically heterogeneous, since no *CASR* mutation can be detected in ~ 10 % of the probands. Two additional loci have been mapped on chromosome 19p13.3 and chromosome 19q13 [240]. It must be noted that patients with autoimmune manifestations may present circulating antibodies to the extracellular domain of the CaSR, which may interfere with the normal activation of the receptor by extracellular Ca^{2+} , leading to acquired FHH with hypocalciuria and hypercalcemia [258].

Table 4 Inherited disorders of the extracellular Ca²⁺-sensing receptor

Disorder	OMIM #	Inheritance	Type of mutation	Age at onset	Serum Ca ²⁺	Serum Mg ²⁺	Serum PTH	Urine Ca ²⁺	Urine Mg ²⁺	Complications
Familial hypocalciuric hypercalcemia (FHH) Familial benign hypercalcemia (FBH)	145980	AD	Loss of function	Childhood	↑	N - ↑	N - ↑	↓	N - ↓	Pancreatitis, chondrocalcinosis, gallstones
Neonatal severe primary hyperparathyroidism (NSHPT)	239200	AR	Loss of function	Neonatal	↑↑	↑	↑↑	↓↓	↓	Life-threatening condition, failure to thrive, osteopenia, fractures
Autosomal dominant hypocalcemia (ADH) Autosomal dominant hypoparathyroidism	146200	AD	Gain of function	Infancy	↓	↓	↓	↑	↑ - ↑↑	Nephrocalcinosis and renal stones under vitamin D treatment Bartter-like syndrome with the most severe activating mutations

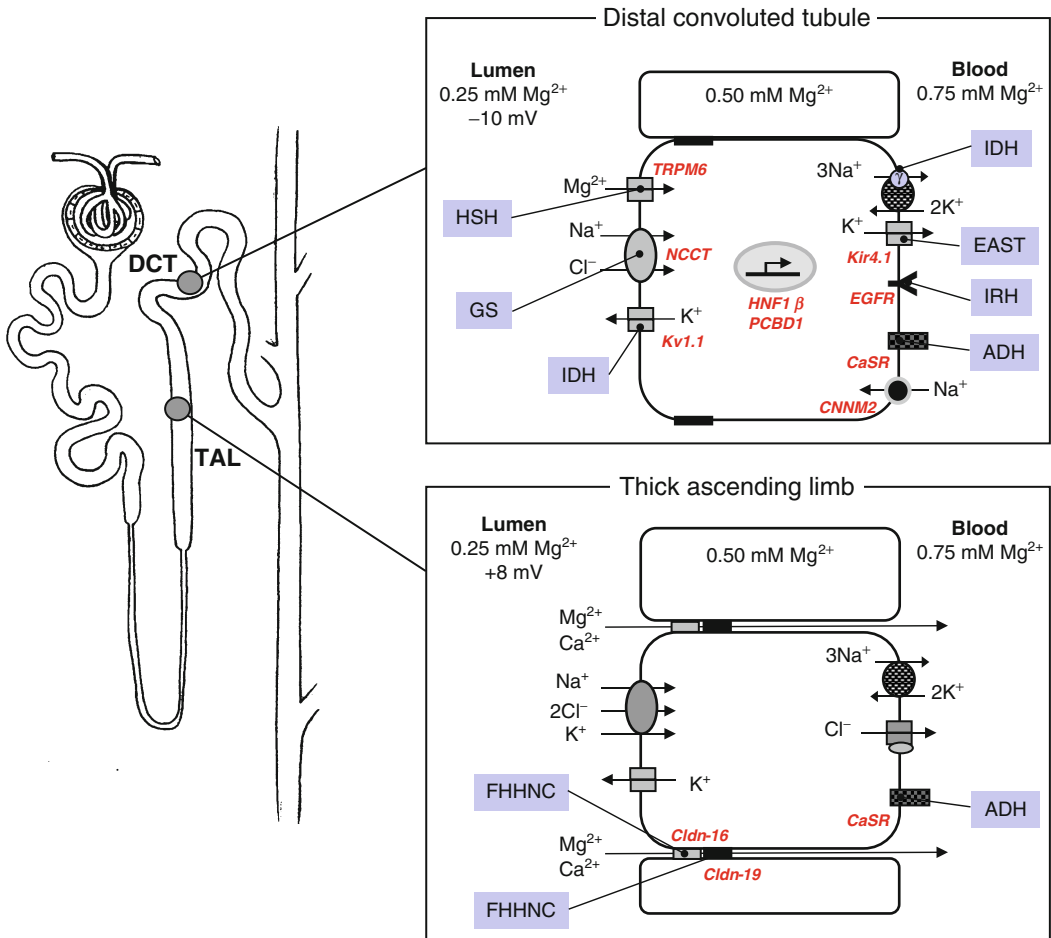


Fig. 3 Inherited disorders of magnesium reabsorption in the loop of Henle and distal convoluted tubule. In the thick ascending limb (TAL) of Henle's loop, Mg²⁺ is reabsorbed through a paracellular pathway, driven by the lumen-positive transcellular voltage generated by the transcellular reabsorption of NaCl. Mutations in the *CLDN16* and *CLDN19* genes that encode the tight junction proteins, claudin-16 and claudin-19, cause familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC). In the distal convoluted tubule (DCT), Mg²⁺ is actively reabsorbed via the transcellular pathway involving an apical entry step through a Mg²⁺-permeable ion channel (TRPM6) and a basolateral exit, presumably mediated by a Na⁺-coupled exchange mechanism. The molecular identity of the basolateral exchange is unknown.

Basolateral EGF stimulates the basolateral EGF receptor (EGFR), which then increases the activity of TRPM6. Mutations of *SLC12A3* coding for NCCT are responsible for Gitelman syndrome (GS). Mutations in the apical TRPM6 channel (*TRPM6*) cause hypomagnesemia with secondary hypocalcemia (HSH), whereas mutations in the γ -subunit of the Na⁺-K⁺-ATPase (*FXYD2*) cause isolated dominant hypomagnesemia (IDH) and mutations in the *EGF* gene coding for the epidermal growth factor (EGF) cause isolated recessive hypomagnesemia (IRH). Loss-of-function mutations in the *CASR* gene (CaSR) are associated with familial hypocalciuric hypercalcemia (FHH) and neonatal severe primary hyperparathyroidism (NSHPT), whereas activating mutations of the CaSR cause autosomal dominant hypocalcemia (ADH)

Neonatal Severe Primary Hyperparathyroidism (NSHPT)

Neonatal severe primary hyperparathyroidism (NSHPT) (OMIM #239200) is a life-threatening,

severe hyperparathyroidism characterized by hypercalcemia, failure to thrive, osteopenia, multiple fractures, and rib cage deformities developing soon after birth [259]. NSHPT is usually caused by homozygous *CASR* mutations in

children born to consanguineous FBH parents [242, 243]. Of note, a marked phenotypic heterogeneity has been observed among four members of a kindred harboring the homozygous (Q164X) mutation of *CASR* [260]. Patients with sporadic NSHPT have been reported to be associated with de novo heterozygous inactivating mutations of *CASR* [240, 253]. At least 60 mutations of the *CASR* gene, mostly missense, have been reported in FHH and NSHPT kindreds (<http://www.hgmd.cf.ac.uk>).

Ho et al. generated a CaSR knockout mouse and showed that the heterozygous mice have modest elevations of serum calcium, magnesium, and parathyroid hormone levels as well as hypocalciuria, thus mimicking FHH, whereas homozygous null mice show markedly elevated serum calcium and parathyroid hormone levels, parathyroid hyperplasia, bone abnormalities, retarded growth, and premature death like humans with NSHPT [261]. In order to remove the confounding effects of elevated PTH and assess the independent function of CaSR, double-homozygous mice lacking CaSR and *Gcm2* were generated [262]. *Gcm2* is the mouse homologue of the *Drosophila gcm* (glial cell missing gene) which is specifically expressed in developing parathyroids, and its genetic ablation in mouse leads to a lack of parathyroid glands [263]. The *Gcm2* deficiency rescued the lethality of CaSR deficiency in this model. Furthermore, the lack of severe hyperparathyroidism prevented rickets and osteomalacia, but it did not rescue the hypocalciuria – indicating that hypocalciuria in FHH and NSHPT is mediated by the lack of CaSR in the kidney [262].

When treating FHH and NSHPT, one should consider that these disorders represent the mildest and severest variants of hyperparathyroidism, respectively. In most kindreds with FHH, the lifelong hypercalcemia is very mild, causing no specific symptoms and requiring no treatment. In contrast, the severe hypercalcemia and hyperparathyroidism associated with NSHPT remain challenging and require specific measures. The acute management of hypercalcemia classically relies on saline perfusion and careful use of loop diuretics. Pamidronate, a bisphosphonate drug

that could halt the bone resorption process mediated by uncontrolled hyperparathyroidism, has been successfully used in NSHPT patients to control severe hypercalcemia prior to parathyroidectomy [260]. Radical subtotal parathyroidectomy is often the treatment of choice in NSHPT [264]. Parathyroidectomy may also be appropriate in kindreds with FHH in which there is unusually severe hypercalcemia, particularly with musculoskeletal and neurobehavioral manifestations, or frankly elevated PTH levels [265]. Calcimimetic CaSR activators, which potentiate the activation of the CaSR by extracellular Ca^{2+} , reset the Ca^{2+} -regulated PTH release in primary and secondary hyperparathyroidism toward normal. These drugs may be of interest in FHH and NSHPT, in which they could increase the sensitivity of the CaSR to extracellular Ca^{2+} , thereby reducing PTH secretion and serum calcium concentration [266]. Such an effect has been documented in a single FHH patient due to a de novo inactivating mutation of the CaSR, in which a maintenance treatment with the calcimimetic drug cinacalcet HCl resulted in a rapid decrease in PTH secretion and a sustained normalization of serum calcium [256].

Autosomal Dominant Hypocalcemia

Activating mutations of the *CASR* gene were first described in families affected with autosomal dominant hypocalcemia (ADH, also named autosomal dominant hypoparathyroidism, or autosomal dominant hypocalcemia with hypercalciuria, ADHH) (OMIM #146200) [243, 244]. Affected individuals present with hypocalcemia, hypercalciuria, and polyuria, and about 50 % of these patients have hypomagnesemia. The serum phosphate concentrations in patients with ADH are either elevated or in the upper normal range [240, 244]. Hypocalcemia in ADH is generally mild to moderate, and patients may present carpopedal spasms and/or seizures. Elevated urinary calcium may lead to nephrolithiasis despite increased magnesium excretion [61].

More than 20 different mutations of the *CASR* gene, mostly missense, have been identified in ADH patients. About half of these mutations are

in the extracellular domain of the CaSR [240]. Expression studies confirmed that these activating mutations induce a leftward shift in the dose–response curve of the mutant CaSR, corresponding to enhanced sensitivity for extracellular Ca^{2+} and Mg^{2+} [244]. This results in inappropriately low serum PTH and decreased reabsorption of Ca^{2+} and Mg^{2+} in the TAL and DCT, leading to Ca^{2+} and Mg^{2+} wasting. The impaired reabsorption of Ca^{2+} and Mg^{2+} in the TAL is thought to be due to a reduction of the paracellular permeability and/or to a decreased lumen-positive transepithelial voltage due to defective transcellular NaCl reabsorption [236]. Hormone-stimulated Mg^{2+} reabsorption is also inhibited in the DCT, which probably contributes to the renal magnesium loss [178].

In treating ADH patients with an activating CaSR mutation, it is important to avoid vitamin D which can dramatically increase urinary calcium excretion, leading to nephrocalcinosis, nephrolithiasis, and even irreversible reduction of renal function in some patients [240, 244]. Therefore, the treatment of hypocalcemia in ADH with vitamin D and calcium supplementation should be restricted to clearly symptomatic patients [61]. Addition of hydrochlorothiazide may reduce urinary calcium excretion and maintain serum calcium concentrations near the lower limit of normal, allowing the reduction of vitamin D treatment [267].

It was shown recently that patients with ADH due to activating *CASR* mutations may have a clinical course complicated with a Bartter-like syndrome, i.e., the development of a salt-losing tubulopathy associated with urinary concentrating defect and hypokalemic metabolic alkalosis [245, 246]. All three patients described thus far had hypomagnesemia. Heterologous expression of the mutant CaSR revealed that the underlying mutations (L125P, C131W, A843E) are among the most severe gain-of-function *CASR* mutations, characterized by a leftward shift in the dose–response curve for the receptor and also a much lower EC50 than patients with ADH [245, 246]. These mutations appear to be fully activated under normal serum Ca^{2+} concentrations and induce a significant salt-losing

phenotype by inhibiting the reabsorption of NaCl in the TAL (Fig. 1). Accordingly, this subset of ADH patients presenting a Bartter-like syndrome was qualified as “type 5 Bartter-like syndrome.” The inclusion of these cases among the Bartter-like syndromes is debated, and it must be pointed that the Bartter-like phenotype may be very mild, as recently reported for another ADH-causing mutation [268].

Disorders of Magnesium Metabolism

Introduction

Magnesium is an important intracellular cation. As a cofactor, it is involved in energy metabolism and protein and nucleic acid synthesis. It is also critical for the modulation of membrane transporters and in signal transduction. Under physiologic conditions, serum Mg^{2+} levels are maintained at almost constant values. Mg^{2+} homeostasis depends on a balanced intestinal absorption and renal excretion. Mg^{2+} deficiency can result from reduced dietary intake, intestinal malabsorption, or renal loss. The control of body Mg^{2+} homeostasis primarily resides in the kidney tubules.

The dietary intake of Mg^{2+} may vary substantially. The principal site of Mg^{2+} absorption is the small intestine, where Mg^{2+} absorption occurs via two different pathways: a saturable active transcellular transport and a nonsaturable paracellular passive transport [269, 270]. In the kidney, approximately 80 % of total serum Mg^{2+} is filtered in the glomeruli, of which more than 95 % is reabsorbed along the nephron. Tubular Mg^{2+} reabsorption differs in quantity and kinetics depending on the different nephron segments. In the adult kidney, approximately 15–20 % is reabsorbed in the PT, whereas the premature kidney of the newborn is able to reabsorb up to 70 % of the filtered Mg^{2+} in this nephron segment [271]. From early childhood on, roughly 70 % of Mg^{2+} is reabsorbed in the cortical TAL of the loop of Henle. Transport in this segment is passive and paracellular, mediated by claudin-16 and claudin-19. The driving force for reabsorption against an unfavorable concentration gradient is the

Table 5 Inherited disorders of renal magnesium handling

Disorder	OMIM #	Inheritance	Gene locus	Gene	Protein
Familial hypomagnesemia with hypercalciuria/nephrocalcinosis	248250	AR	3q28	<i>CLDN16</i>	Claudin-16 (paracellin-1), tight junction protein
Familial hypomagnesemia with hypercalciuria/nephrocalcinosis and ocular involvement	248190	AR	1p34	<i>CLDN19</i>	Claudin-19, tight junction protein
Gitelman syndrome	263800	AR	16q13	<i>SLC12A3</i>	NCCT, Na ⁺ -Cl ⁻ cotransporter
EAST/SeSAME syndrome	612780	AR	1q23	<i>KCNJ10</i>	Kir4.1, potassium channel
Isolated dominant hypomagnesemia	154020 176260	AD AD	11q23 12p13	<i>FXYD2</i> <i>KCNA1</i>	γ-Subunit of the Na ⁺ -K ⁺ -ATPase Kv1.1, apical potassium channel
Isolated recessive hypomagnesemia	611718	AR	4q25	<i>EGF</i>	Pro-EGF, epidermal growth factor
Hypomagnesemia with secondary hypocalcemia	602014	AR	9q22	<i>TRPM6</i>	TRPM6, Mg ²⁺ channel
Hypomagnesemia with impaired brain development	613882	AD/AR	10q24	<i>CNNM2</i>	Cyclin M2
HNF1B nephropathy	137920	AD	17q12	<i>HNF1B</i>	HNF1beta, transcription factor
Hypomagnesemia after transient neonatal hyperphenylalaninemia	264070	AR	10q22	<i>PCBD1</i>	PCBD1, tetrahydrobiopterin metabolism
Hypomagnesemia/metabolic syndrome	500005	Maternal	mtDNA	<i>MTTI</i>	Mitochondrial tRNA (isoleucin)

lumen-positive transepithelial voltage (Fig. 3). Only 5–10 % of the filtered Mg²⁺ is reabsorbed in the DCT. However, in this part of the nephron, the fine adjustment of renal excretion is accomplished. In the DCT, Mg²⁺ transport is an active transcellular process (Fig. 3). Physiologic studies indicate that apical entry into DCT cells is mediated by the specific and regulated Mg²⁺ channel TRPM6. The mechanism of basolateral transport into the interstitium is unknown. Here, Mg²⁺ has to be extruded against an unfavorable electrochemical gradient. Most physiologic studies favor a Na⁺-dependent exchange mechanism [272]. Mg²⁺ entry into DCT cells appears to be the rate-limiting step and the site of regulation. For details of Mg²⁺ transport in the distal tubule, see Dai et al. [178]. In the collecting duct, there is no significant Mg²⁺ uptake. Finally, 3–5 % of the filtered Mg²⁺ is excreted in the urine.

Magnesium depletion is usually secondary to another disease process or to a therapeutic agent (e.g., loop diuretics, thiazides, aminoglycosides, cisplatin, calcineurin inhibitors, proton pump inhibitors, EGF receptor antibodies). During infancy and childhood, a substantial proportion of patients receiving medical attention for signs of hypomagnesemia are affected by inherited renal disorders associated with Mg²⁺ wasting. In these disorders, hypomagnesemia either may be a leading symptom or may be part of a complex phenotype resulting from tubular dysfunction, as will be detailed below. Recent advances in molecular genetics of hereditary hypomagnesemia substantiated the role of a variety of genes and their encoded proteins in human epithelial Mg²⁺ transport and helped to characterize different clinical subtypes of hereditary Mg²⁺ wasting (Table 5). A careful clinical and biochemical assessment

Table 6 Clinical and biochemical characteristics of inherited hypomagnesemia

Disorder	Age at onset	Serum Mg ²⁺	Serum Ca ²⁺	Serum K ⁺	Blood pH	Urine Mg ²⁺	Urine Ca ²⁺	Nephrocalcinosis	Renal stones
Familial hypomagnesemia with hypercalciuria/nephrocalcinosis	Childhood	↓	N	N	N or ↓	↑↑	↑↑	Yes	Yes
Gitelman syndrome	Adolescence	↓	N	↓	↑	↑	↓	No	No
EAST/SeSAME syndrome	Infancy	↓	N	↓	↓	↑	↓	No	No
Isolated dominant hypomagnesemia	Childhood	↓	N	N	N	↑	↓	No	No
Isolated recessive hypomagnesemia	Childhood	↓	N	N	N	↑	N	No	No
Hypomagnesemia with secondary hypocalcemia	Infancy	↓↓↓	↓	N	N	↑	N	No	No
Hypomagnesemia with impaired brain development	Broad range	↓↓	N	N	N	↑	?	No	No
HNF1B nephropathy	Broad range	↓	N	N	N	↑	↓	No	No
Hypomagnesemia after transient neonatal hyperphenylalaninemia	Adulthood	↓	N	N	?	↑	N	No	No
Hypomagnesemia/metabolic syndrome	Adulthood	↓	N	N	N	↑	↓	No	No

allows to distinguish the different disease entities in most cases, even when there is a considerable overlap in the phenotypic characteristics (Table 6).

Familial Hypomagnesemia with Hypercalciuria and Nephrocalcinosis

Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC, OMIM #248250) is an autosomal recessive disorder caused by mutations in two different members of the claudin gene family, namely, claudin-16 and claudin-19 [273, 274]. Since its first description by Michelis et al. [275], more than 150 patients have been reported allowing a comprehensive characterization of the clinical spectrum [276–285]. As a consequence of excessive renal Mg²⁺ and Ca²⁺ wasting, patients develop the characteristic triad of hypomagnesemia, hypercalciuria, and nephrocalcinosis. Additional laboratory findings include elevated PTH levels before the onset of chronic renal failure,

incomplete distal tubular acidosis, hypocitraturia, and hyperuricemia, which are present in most patients [281]. Typically, FHHNC patients clinically present during early childhood with recurrent urinary tract infections, polyuria/polydipsia, nephrolithiasis, and/or failure to thrive. Clinical signs of severe hypomagnesemia are less common. The clinical course of FHHNC is frequently complicated by the development of chronic renal failure (CRF) during the first two decades of life. A considerable number of patients exhibit a marked decline in GFR (<60 ml/min per 1.73 m²) already at the time of diagnosis, and about one third of patients develop ESRD during adolescence. Due to a reduction in filtered Mg²⁺ that limits urinary Mg²⁺ losses, hypomagnesemia may completely disappear with the decline of GFR.

Whereas the renal phenotype is almost identical in carriers of *CLDN16* and *CLDN19* mutations, ocular involvement is only in patients with *CLDN19* mutations [274, 279, 284, 285].

By positional cloning, Simon et al. first identified one gene, namely, *CLDN16* (formerly known

as *PCLN1*), which is mutated in many patients with FHHNC [273]. *CLDN16* codes for claudin-16, a member of the claudin family. More than 20 claudins identified so far comprise a family of ~22kD proteins with four transmembrane segments, two extracellular domains, and intracellular N and C termini. Claudins are important components of tight junctions. The individual composition of tight junction strands with different claudin members confers the characteristic properties of different epithelia regarding paracellular permeability and/or transepithelial resistance. In this context, a crucial role has been attributed to the first extracellular domain of the claudin protein which is extremely variable in number and position of charged amino acid residues. Individual charges have been shown to influence paracellular ion selectivity, suggesting that claudins positioned on opposing cells forming the paracellular pathway provide charge-selective pores within the tight junction barrier.

Most mutations reported so far in FHHNC are simple missense mutations affecting the transmembrane domains and the extracellular loops with a particular clustering in the first extracellular loop which contains the ion selectivity filter. Within this domain, patients originating from Germany or Eastern European countries exhibit a common *CLDN16* mutation (L151F) due to a founder effect [281]. As this mutation is present in approximately 50 % of mutant alleles, molecular diagnosis is greatly facilitated in patients originating from these countries.

Defects in *Cldn16* have also been shown to underlie the development of a chronic interstitial nephritis in Japanese cattle that rapidly develop chronic renal failure shortly after birth [286, 287]. Interestingly, affected animals show hypocalcemia but no hypomagnesemia, which might be explained by advanced renal failure present at the time of examination. The fact that, in contrast to the point mutations identified in human FHHNC, large deletions of *Cldn16* are responsible for the disease in cattle might explain the more severe phenotype with early-onset renal failure. However, *Cldn16* knockout mice do not display renal failure during the first months of life [288].

In FHHNC patients, progressive renal failure is generally thought to be a consequence of massive urinary Ca^{2+} wasting and nephrocalcinosis. A study of a large cohort of FHHNC patients showed that the presence of *CLDN16* mutations leading to a complete loss-of-function on both alleles displays a younger age at manifestation as well as a more rapid decline in renal function compared to patients with at least one allele with residual claudin-16 function (2283). These findings support the idea that a complete lack of claudin-16 is associated with a more severe phenotype, whereas a residual function delays the progression of renal failure.

Two independent studies described a high incidence of hypercalciuria, nephrolithiasis, and/or nephrocalcinosis in first-degree relatives of FHHNC patients [279, 281]. A subsequent study also reported a tendency toward mild hypomagnesemia in family members with heterozygous *CLDN16* mutations [289]. Thus, one might speculate that *CLDN16* mutations could be involved in idiopathic hypercalciuric stone formation.

A homozygous *CLDN16* mutation (T303R) affecting the C-terminal PDZ domain has been identified in two families with isolated hypercalciuria and nephrocalcinosis without disturbances in renal Mg^{2+} handling [290]. Interestingly, the hypercalciuria disappeared during follow-up and urinary Ca^{2+} levels reached normal values beyond puberty.

Molecular genetic studies in FHHNC patients with severe ocular involvement subsequently lead to the identification of mutations in a second member of the claudin family, namely, claudin-19 (encoded by *CLDN19*) [283]. The identification of *CLDN19* mutations could explain the variable ocular phenotype observed in FHHNC, because *CLDN19* defects seem to be invariably associated with severe ocular abnormalities (including severe myopia, nystagmus, or macular coloboma) [278–280]. The latter association has been named FHHNC with severe ocular involvement (OMIM #248190). In contrast, only a small subset of FHHNC patients with *CLDN16* defects display severe myopia, whereas nystagmus or colobomata have not been described [281]. The renal phenotype is very similar between these two

FHHNC subtypes. Expression studies revealed that claudin-16 and claudin-19 perfectly colocalize at tight junctions of the TAL [274]. It could further be demonstrated that claudin-16 and claudin-19 functionally interact, which could increase the cation selectivity of tight junctions above that of claudin-16 alone [291]. This is most likely due to anion-blocking properties of claudin-19 preventing back diffusion of Cl^- anions to the tubular lumen.

Besides continuous oral Mg^{2+} supplementation, therapy aims to reduce Ca^{2+} excretion in order to prevent the progression of nephrocalcinosis and stone formation because the degree of renal calcifications has been correlated with progression of chronic renal failure [279]. In the short-term study, thiazides have been demonstrated to effectively reduce urinary Ca^{2+} excretion in FHHNC patients [282]. However, these therapeutic strategies have not been shown yet to significantly influence the progression of renal failure. Supportive therapy is important for the protection of kidney function and should include provision of sufficient fluids and effective treatment of stone formation and bacterial colonization. Renal transplantation does not result in recurrence of the disease because the primary defect resides in the kidney.

Gitelman Syndrome

This primary salt-wasting disorder complicated by urinary Mg^{2+} wasting and hypomagnesemia is discussed in detail above.

EAST/SeSAME Syndrome

In 2009, a newly characterized clinical syndrome with autosomal recessive inheritance combining epilepsy, ataxia, sensorineural deafness, and renal NaCl wasting with/without mental retardation was described under the acronyms EAST or SeSAME syndrome (OMIM #612780) [292, 293]. Patients usually present early in infancy with generalized tonic-clonic seizures, speech and motor delay, as well as severe ataxia leading to an

inability to walk, intention tremor, and dysidiadochokinesia. In addition they exhibit a severe hearing impairment. Renal salt wasting may develop or be recognized only later during the course of the disease [294]. Closely resembling GS, the renal phenotype includes the combination of hypokalemic alkalosis, hypomagnesemia, and hypocalciuria.

EAST/SeSAME syndrome is caused by loss-of-function mutations in the *KCNJ10* gene encoding the inwardly rectifying K^+ channel Kir4.1 [292, 293]. The expression pattern of Kir4.1 fits to the disease phenotype with high expression levels in the brain, the stria vascularis of the inner ear, and the distal nephron, especially in the DCT. Here, Kir4.1 is localized at the basolateral membrane of DCT cells (Fig. 3) and supposed to function in collaboration with Na^+-K^+ -ATPase as it might allow for a recycling of K^+ ions entering the tubular cells in countermove for the extruded Na^+ [293]. Loss of Kir4.1 function most likely leads to a depolarization of the basolateral membrane and thereby to a reduction of the driving force for basolateral anion channels as well as sodium-coupled exchangers. By this mechanism, Kir4.1 defects could also affect the putative $\text{Na}^+/\text{Mg}^{2+}$ exchanger and possibly explain the Mg^{2+} wasting observed in EAST/SeSAME syndrome. Moreover, it could be demonstrated that lack of Kir4.1 decreases basolateral Cl^- conductance and results in a diminished expression of NCCT in the apical membrane [295]. These results could explain the salt loss observed in EAST/SeSAME patients. Interestingly, the renal phenotype of *Kir4.1*^{-/-} mice had not been thoroughly studied until the description of human disease [296]. The reevaluation of *Kir4.1*^{-/-} mice by Bockenhauer and colleagues however clearly demonstrated renal salt wasting leading to significant growth retardation [292].

Isolated Dominant Hypomagnesemia

A first variant of isolated dominant hypomagnesemia (IDH, OMIM #154020) was described in two related families by Meij et al. who discovered

a missense mutation in the *FXYD2* gene which encodes a γ -subunit of the $\text{Na}^+\text{-K}^+\text{-ATPase}$ [297]. The index patients presented with seizures during childhood (at 7 and 13 years) with serum Mg^{2+} levels of approximately 0.4 mmol/L. One patient was treated for seizures of unknown origin with antiepileptic drugs until serum Mg^{2+} levels were evaluated during adolescence. At that time mental retardation was evident [298, 299]. Serum Mg^{2+} measurements performed in additional members of both families revealed low serum Mg^{2+} levels in numerous apparently healthy individuals. A ^{28}Mg -retention study in one of the patients indicated a primary renal defect [298]. The intestinal absorption of Mg^{2+} was preserved and even stimulated in compensation for the increased renal losses. Urinary Mg^{2+} measurements in affected family members revealed significant renal Mg^{2+} loss (around 5 mmol per day) despite profound hypomagnesemia. Urinary Ca^{2+} excretion rates were low in all hypomagnesemic individuals, a finding reminiscent of patients presenting with GS. However, in contrast to GS patients, no other biochemical abnormalities were reported, especially no hypokalemic alkalosis.

The protein encoded by *FXYD2* is a member of a small single transmembrane protein family which shares the common amino acid motif F-X-Y-D. *FXYD* proteins modulate the function of the ubiquitous $\text{Na}^+\text{-K}^+\text{-ATPase}$, a dimeric enzyme invariably consisting of one α - and one β -subunit (Fig. 3). *FXYD* proteins constitute a third or γ -subunit that represents a tissue-specific regulator of the $\text{Na}^+\text{-K}^+\text{-ATPase}$. Two members of this family, *FXYD2* and *FXYD4*, are highly expressed along the nephron displaying an alternating expression pattern [300]. The *FXYD2* γ -subunit comprises two isoforms (named γ - α and γ - β) that are differentially expressed in the kidney. The γ - α -isoform is present predominantly in the proximal tubule, and expression of the γ - β -isoform predominates in the distal nephron, especially in the DCT and connecting tubule [301]. The *FXYD2* γ -subunit increases the apparent affinity of $\text{Na}^+\text{-K}^+\text{-ATPase}$ for ATP, while decreasing its Na^+ affinity [302]. Thus, it might provide a mechanism for balancing energy utilization and maintaining appropriate salt gradients.

Expression studies of the mutant G41R- γ -subunit revealed a dominant-negative effect leading to a retention of the γ -subunit in the Golgi complex. The mechanism of a dominant-negative effect is supported by the observation that individuals with a large heterozygous deletion of chromosome 11q including the *FXYD2* gene exhibit normal serum Mg^{2+} levels [299]. Urinary Mg^{2+} wasting and the expression pattern of the *FXYD2* gene indicate defective transcellular Mg^{2+} reabsorption in the DCT. The exact mechanism causing increased urinary Mg^{2+} excretion has yet to be determined. Meij and colleagues have suggested that diminished intracellular K^+ might depolarize the apical membrane resulting in a decreased Mg^{2+} uptake [297]. Alternatively, an increase in intracellular Na^+ could impair basolateral Mg^{2+} transport which is presumably achieved by a Na^+ -coupled exchange mechanism. Another explanation could be that the γ -subunit is not only involved in $\text{Na}^+\text{-K}^+\text{-ATPase}$ function but also an essential component of a yet unidentified ATP-dependent transport system specific for Mg^{2+} . Similar to Ca^{2+} , both a specific $\text{Mg}^{2+}\text{-ATPase}$ and a Na^+ -coupled exchanger might exist. Further studies are needed to clarify this issue.

An interesting clinical feature of IDH related to the *FXYD2* defect is the finding of hypocalciuria which is primarily observed in GS. Unfortunately, only one large family has been described and an animal model carrying the human mutation is not available. Mice lacking the γ -subunit (*Fxyd2*) do not demonstrate significant abnormalities in Mg^{2+} conservation or balance [303]. One could speculate that, like in GS, a defect in $\text{Na}^+\text{-K}^+\text{-ATPase}$ function and energy metabolism might lead to an apoptotic breakdown of the early DCT responsible for Mg^{2+} reabsorption, while later parts of the distal nephron remain intact. In IDH patients with *FXYD2* defects, there is no evidence for renal salt wasting and no stimulation of the RAAS. The finding of hypocalciuria without apparent volume depletion apparently contradicts recent experimental data which favor an increase in proximal tubular Ca^{2+} reabsorption due to volume depletion in GS.

Genetic heterogeneity in IDH (OMIM#176260) was demonstrated by the

identification of a heterozygous missense mutation in the *KCNA1* gene which encodes the voltage-gated potassium channel Kv1.1 [304]. The observed clinical picture includes early-onset muscle cramps, tetany, tremor, and muscle weakness. Biochemical workup revealed a renal Mg^{2+} leak without alterations in renal Ca^{2+} handling. Mutations in *KCNA1* had previously been identified in patients with episodic ataxia with myokymia (OMIM 160120), a neurologic disorder characterized by an intermittent appearance of incoordination and imbalance as well as myokymia, an involuntary, spontaneous, and localized trembling of muscles. In addition to muscle cramps and tetany attributed to Mg^{2+} deficiency, these symptoms were also present in members of the aforementioned family with hypomagnesemia.

The mutation in *KCNA1* described in hypomagnesemic patients leads to a nonconservative amino acid exchange (N255D) in the Kv1.1 K^+ channel. Functional voltage-gated K^+ channels of the KCNA family are composed of tetramers. Co-expression of the N255D mutant with wild-type Kv1.1 subunits revealed a dominant-negative effect that rather seems to result from impaired channel gating since trafficking to the plasma membrane is preserved [305].

Kv1.1 expression was demonstrated in DCT cells, presumably at the apical membrane (Fig. 3). Because of its colocalization with TRPM6, a model was proposed in which Kv1.1 allows for hyperpolarization of the apical membrane of DCT cells as a prerequisite for TRPM6-mediated Mg^{2+} entry (Fig. 3).

Isolated Recessive Hypomagnesemia

Geven and colleagues reported a form of isolated recessive hypomagnesemia (IRH, OMIM #611718) in a consanguineous family [306]. Two affected girls presented with generalized seizures during infancy. Possibly related to late diagnosis, both patients also exhibited neurodevelopmental deficits. Clinical and laboratory workup at 4 and 8 years of age, respectively, revealed serum Mg^{2+} levels around 0.5–0.6

mmol/L with no other associated electrolyte abnormalities. A ^{28}Mg -retention study in one patient pointed to a primary renal defect, while intestinal Mg^{2+} uptake was preserved [306]. Both patients exhibited renal Mg^{2+} excretion of 3–6 mmol per day despite hypomagnesemia confirming renal Mg^{2+} wasting. Renal Ca^{2+} excretion rates in IRH are within the normal range.

The molecular defect for IRH was identified by Groenestege et al. who demonstrated a homozygous P1070L mutation in both affected siblings in the *EGF* gene encoding the epidermal growth factor (EGF) [307]. The EGF protein is expressed in the DCT, and its binding to the EGF receptor (EGFR) is essential for the function of the TRPM6 channel (Fig. 3). The mutation is located in the cytosolic C-terminal terminus within a sorting motif (PXXP) which is necessary for the trafficking of EGF to the basolateral membrane. Expression studies demonstrated that mutant pro-EGF retains EGF secretion to the apical membrane but does not reach the EGF receptor in the basolateral membrane, resulting in dysfunction of TRPM6 [307]. Even if IRH seems to be an extremely rare disease phenotype, the identification of EGF mutations is important because this is the first autocrine/paracrine magnesiotropic hormone known at the molecular level.

Hypomagnesemia with Secondary Hypocalcemia

Hypomagnesemia with secondary hypocalcemia (HSH, OMIM #602014) is a rare autosomal recessive disorder first described in 1968 [308]. It manifests in early infancy with generalized seizures or other symptoms of increased neuromuscular excitability. Biochemical abnormalities include extremely low serum Mg^{2+} (about 0.2 mmol/L) and low serum Ca^{2+} levels. The mechanism leading to hypocalcemia is still not completely understood. Severe hypomagnesemia results in an impaired synthesis and/or release of PTH [309]. Consistently, PTH levels in HSH patients were found to be inappropriately low. The hypocalcemia observed in HSH does not respond to therapy with Ca^{2+} or vitamin D. Relief of clinical

symptoms, normocalcemia, and normalization of PTH levels can only be achieved by administration of high doses of Mg^{2+} [310]. Delayed diagnosis or noncompliance with treatment can be fatal or result in permanent neurological damage.

Transport studies in HSH patients indicated a primary defect in intestinal Mg^{2+} absorption [311]. However, in some patients, an additional renal leak for Mg^{2+} was suspected [312]. A gene locus (*HOMG1*) for HSH had been mapped to chromosome 9q22 in 1997 [313]. Later, two independent groups identified *TRPM6* at this locus and reported loss-of-function mutations as the underlying cause of HSH [179, 314]. To date, mutations in *TRPM6* have been identified in more than 40 families affected by HSH [179, 314–318]. Whereas the majority of HSH mutations are nonsense, frameshift, and splice site mutations or small deletions which all lead to a truncated TRPM6 protein, only few missense mutations have been reported [179, 314, 318, 319]. Functional data for a subset of these also indicate a complete loss of function which might therefore be considered as a prerequisite for the development of the typical HSH phenotype.

TRPM6 encodes a member of the transient receptor potential (TRP) family of cation channels (Fig. 3). The TRPM6 protein is homologous to TRPM7, a Ca^{2+} and Mg^{2+} permeable ion channel regulated by Mg-ATP [320]. TRPM6 is expressed along the entire small intestine and colon but also in the kidney in distal tubule cells. Immunofluorescence studies localized TRPM6 to the apical membrane of the DCT [321] confirming that renal Mg^{2+} wasting could play a role in the pathogenesis of HSH [322]. This was also supported by intravenous Mg^{2+} loading tests in HSH patients, which disclosed a considerable renal Mg^{2+} leak [314].

TRPM6 is closely related to TRPM7 and represents the second TRP protein being fused to a C-terminal α -kinase domain. The *TRPM6* gene encodes a large protein with 2022-amino-acid residues. *TRPM6* mRNA shows a more restricted expression pattern than TRPM7 with highest levels along the intestine and the DCT of the kidney [179]. Immunohistochemistry shows a complete colocalization with the Na^+-Cl^-

cotransporter NCCT (also serving as a DCT marker) but also with parvalbumin and calbindin- D_{28K} , two cytosolic proteins that putatively act as intracellular (Ca^{2+} and) Mg^{2+} buffers [321]. As yet, the biophysical characterization of TRPM6 remains controversial. Voets et al. could demonstrate striking parallels between TRPM6 and TRPM7 with respect to gating mechanisms and ion selectivity profiles, since TRPM6 was shown to be regulated by intracellular Mg^{2+} levels and to be permeable for Mg^{2+} and Ca^{2+} [321]. Permeation characteristics with currents almost exclusively carried by divalent cations with a higher affinity for Mg^{2+} than Ca^{2+} support the role of TRPM6 as the apical Mg^{2+} influx pathway. Furthermore, TRPM6 – analogous to TRPM7 – exhibits a marked sensitivity to intracellular Mg^{2+} . Thus one might speculate about an inhibition of TRPM6-mediated Mg^{2+} uptake by rising intracellular Mg^{2+} concentrations, as a possible mechanism for regulation of intestinal and renal Mg^{2+} (re)absorption. This inhibition might in part be mediated by intracellular Mg-ATP as shown for TRPM7 [320]. Chubanov et al. reported that TRPM6 is only present at the cell surface when associating with TRPM7 [323]. Furthermore, FRET (fluorescence resonance energy transfer) analyses showed a specific direct protein–protein interaction between both proteins. Electrophysiological data in a *Xenopus* oocyte expression system indicated that co-expression of TRPM6 results in a significant amplification of TRPM7-induced currents [323]. Schmitz et al. [324] demonstrated that TRPM6 and TRPM7 are not functionally redundant and that both proteins can influence each other's biological activity. In particular, TRPM6 can phosphorylate TRPM7 and TRPM6 might modulate TRPM7 function in a Mg^{2+} -dependant manner [324].

TRPM6 has been shown to be regulated by the first magnesiotropic hormone identified so far, namely, the epithelial growth factor (EGF) which is expressed in the DCT. In a cell culture model, Groenstege et al. could show that EGF increases the activity of TRPM6 expressing cells [307]. This is in line with the clinical observation that cancer patients treated with cetuximab, a monoclonal antibody directed against the EGF

receptor, develop hypomagnesemia secondary to increased Mg^{2+} wasting. These findings suggest that EGF acts in an autocrine or a paracrine manner to stimulate TRPM6 activity leading to increased reabsorption of Mg^{2+} in the DCT. TRPM6 is also regulated by changes in body magnesium content, with hypomagnesemia resulting in the upregulation of TRPM6 expression not only in the DCT but also in the gastrointestinal tract [325]. Similarly, 17-beta-estradiol induces an upregulation of TRPM6, as shown in ovariectomized rats [325]. From a clinical point of view, it is important to note that the well-known hypomagnesemia in patients receiving calcineurin inhibitors (cyclosporin A, FK506) is at least in part mediated by downregulation of TRPM6 [326, 327].

Hypomagnesemia with Impaired Brain Development

Another form of hereditary Mg^{2+} wasting could be linked to mutations in *CNNM2* encoding the transmembrane protein CNNM2 or cyclin M2 (OMIM # 613882) [328]. *CNNM2* (originally termed ACDP2) had previously been identified by differential expression in a mouse DCT cell line under varying Mg^{2+} concentrations as well as by microarray analysis of the renal transcriptome in mice lacking claudin-16 [288, 329]. In addition, common variants in *CNNM2* had been shown to be associated with serum Mg^{2+} levels in a genome-wide association study [330].

Stuiver et al. were able to identify heterozygous *CNNM2* mutations in two families with autosomal dominant hypomagnesemia [328]. Clinical signs and age at manifestation were variable with symptoms ranging from seizures in early childhood to muscle weakness, vertigo, and headache during adolescence. Other heterozygous carriers from both families even remained asymptomatic. Except for hypomagnesemia (appr. 0.4–0.5 mmol/L), no additional serum or urine electrolyte abnormalities were described. Whereas a truncating frameshift mutation was identified in one of the described families, affected individuals of the second family were found to carry a missense

mutation leading to a nonconservative amino acid exchange in CNNM2. The functional characterization of the second mutations (T568I) demonstrated that the protein trafficking in HEK293 cells was preserved. However, patch clamp analyses revealed a significant reduction in Mg^{2+} -sensitive, inwardly rectifying Na^+ currents [328].

The phenotypic and genetic spectrum of *CNNM2* defects was broadened by the identification of a recessive mutation in a family with parental consanguinity [331]. The two affected siblings presented during infancy with cerebral convulsions resistant to conventional anticonvulsive therapy. Diagnostic workup revealed hypomagnesemia (~0.5 mmol/L) and a severe degree of psychomotor retardation. MR imaging of the central nervous system in one of the patients showed widened outer cerebrospinal liquor spaces and myelination defects. By screening a larger cohort of sporadic hypomagnesemia cases, the same study revealed four additional heterozygous de novo mutations meaning that they were not detected in the parental lineage. The affected children presented in infancy with generalized convulsions and displayed a significant degree of intellectual disability [331].

Despite this clinical and genetic progress, the precise physiological function of CNNM2 still remains enigmatic. CNNM2 is ubiquitously expressed in mammalian tissues, most prominently in the kidney, brain, and lung [332, 333]. In the kidney, CNNM2 expression was demonstrated at the basolateral membrane of TAL and DCT (Fig. 3).

Although CNNM2 had been proposed as a Mg^{2+} transporter after overexpression studies in *Xenopus* oocytes [329], Mg^{2+} transport could not be directly measured in mammalian cells using patch clamp analyses [305]. In silico modeling of CNNM2 protein domains identified a Mg^{2+} -ATP binding site suggesting a putative role in Mg^{2+} sensing [333]. Using stable Mg^{2+} isotopes, an increase in cellular Mg^{2+} uptake could be demonstrated after overexpression of CNNM2, an effect that was abrogated by the introduction of pathogenic mutations identified in hypomagnesemic patients [331]. Finally, CNNM2 function was studied in the zebra fish

model. Here, knockdown of *CNNM2* expression leads to a decrease in whole-body Mg^{2+} as well as developmental defects of the central nervous system in line with human disease. The zebra fish phenotype could be rescued by wild-type mammalian *Cnm2*, but not by *Cnm2* with inserted human mutations underlining the specificity of the knockdown approach [331]. Further studies will be needed to clarify if *CNNM2* serves as a Mg^{2+} sensor or if it by itself is able to transport Mg^{2+} .

HNF1B Nephropathy

Hepatocyte nuclear factor 1 β (HNF1B) is a transcription factor which plays a critical role for the development of the kidney and the pancreas. Heterozygous mutations in *HNF1B* were initially reported in maturity-onset diabetes of the young (MODY5) [334]. Subsequent reports described an association with developmental renal disease. The kidney anomalies are highly variable comprising enlarged hyperechogenic kidneys, multicystic kidney disease, renal agenesis, renal hypoplasia, cystic dysplasia, as well as hyperuricemic nephropathy. The association of MODY and cystic kidneys led to the term renal cysts and diabetes syndrome (RCAD) [335]. However, this denomination might be misleading because neither the renal cystic phenotype nor the diabetes is constant clinical findings [336]. For this reason, the new term HNF1B nephropathy (OMIM# 137920) has been introduced.

HNF1B mutations occur in the heterozygous state, either inherited or de novo, and comprise point mutations as well as whole gene deletions [337]. Approximately 50 % of patients develop hypomagnesemia due to impaired renal Mg^{2+} conservation [338]. Renal Mg^{2+} wasting is accompanied by hypocalciuria indicating an involvement of the DCT.

HNF1B encodes a transcription factor of the homeodomain-containing superfamily that regulates the expression of numerous renal genes including *FXYD2* (see above; Fig. 3), which contains several HNF1B-binding sites in the promoter region [338]. Adalat et al. demonstrated that HNF1B can induce the expression of

FXYD2 in vitro [338]. Therefore, defective *FXYD2* transcription potentially represents a mechanism explaining renal Mg^{2+} wasting in patients with *HNF1B* mutations.

Transient Neonatal Hyperphenylalaninemia

Renal Mg^{2+} wasting has recently been described in transient neonatal hyperphenylalaninemia due to recessive mutations in the *PCBD1* gene (OMIM# 264070) [339]. Affected patients were shown to develop hypomagnesemia and a MODY-type diabetes at adult age. Functional studies revealed that PCBD1 is an essential dimerization cofactor of HNF1B. It was demonstrated that a defective dimerization of PCBD1 with HNF1B abrogated the HNF1B-mediated stimulation of *FXYD2* promoter activity in the DCT [339].

Mitochondrial Hypomagnesemia

A mutation in the mitochondrially encoded isoleucine tRNA gene, tRNA^{Ile} or MTTI, related to hypomagnesemia has been discovered in a large Caucasian kindred [340]. An extensive clinical evaluation of this family was prompted after the discovery of hypomagnesemia in the index patient, leading to the characterization of mitochondrial hypomagnesemia (OMIM #500005). Indeed, pedigree analysis was compatible with mitochondrial inheritance as the phenotype was exclusively transmitted by affected females. The phenotype includes hypomagnesemia, hypercholesterolemia, and hypertension. Of the adults on the maternal lineage, the majority of offspring exhibited at least one of the mentioned symptoms, approximately half of the individuals showed a combination of two or more symptoms, and around 1/6 had all three features. Serum Mg^{2+} levels of family members on the maternal lineage varied greatly from ~0.3 to ~1.0 mmol/L with approximately 50 % of individuals being hypomagnesemic. The hypomagnesemic individuals (serum Mg^{2+} < 0.9 mmol/L) showed higher

fractional excretions (median around 7.5 %) than their normomagnesemic relatives (median around 3 %) clearly pointing to renal Mg^{2+} wasting as causative for hypomagnesemia. Interestingly, hypomagnesemia was accompanied by decreased urinary Ca^{2+} levels, a finding pointing to the DCT as the affected tubular segment.

The observed nucleotide change of the MTTI gene occurs at the T-nucleotide directly adjacent to the anticodon triplet. This position is highly conserved among species and critical for codon–anticodon recognition. The functional consequences of the tRNA defect for mitochondrial function remain to be elucidated in detail. As ATP consumption along the tubule is highest in the DCT, the authors speculate about an impaired energy metabolism of DCT cells as a consequence of the mitochondrial defect which in turn could lead to disturbed transcellular Mg^{2+} reabsorption [340]. Further studies in these patients might help to better understand the mechanism of distal tubular Mg^{2+} wasting in this disease.

Management of Hypomagnesemia/ Magnesium Deficiency

The main goal of Mg^{2+} substitution in hypomagnesemic patients is the relief of clinical symptoms. In most cases, especially in primary Mg^{2+} wasting diseases, normal levels cannot be achieved by oral substitution without considerable gastrointestinal side effects. The route of administration depends on the severity of clinical symptoms. Acute intravenous infusions should be reserved for patients with severe symptoms, i.e., with cerebral seizures [341]. Especially in children, painful intramuscular injections should be avoided. In infants and children, the starting dose is 20–50 mg Mg^{2+} sulfate (0.1–0.2 mmol Mg^{2+}) per kilogram body weight. Mg^{2+} sulfate should be given slowly intravenously (over 20 min). The maximum dose for adults is 2 g of Mg^{2+} sulfate. Single doses can be repeated every 6–8 h or followed by continuous infusion of 100–200 mg Mg^{2+} sulfate (0.4–0.8 mmol Mg^{2+}) per kilogram of body weight per day [342]. During Mg^{2+} infusion, close monitoring of cardiorespiratory

function is important and Ca^{2+} gluconate should be available as an antidote. The assessment of renal function is also mandatory.

Asymptomatic hypomagnesemia or chronic Mg^{2+} deficiency should be treated with oral Mg^{2+} substitution. In children, 10–20 mg Mg^{2+} (0.4–0.8 mmol) per kg of body weight given three to four times a day has been recommended to correct hypomagnesemia [343]. Of note, the solubility, intestinal absorption, and side effects considerably differ depending on the Mg^{2+} salt used for oral therapy. The bioavailability and pharmacokinetics of different Mg^{2+} salts have been reviewed recently [344]. With respect to solubility, intestinal absorption, and bioavailability, organic Mg^{2+} salts (e.g., citrate or aspartate) appear most suitable for oral substitution. Moreover, the laxative effect of organic Mg^{2+} salts seems to be less pronounced compared to inorganic salts.

The use of certain diuretics has been proposed for the reduction of renal Mg^{2+} excretion. Both K^+ -sparing diuretics and aldosterone antagonists exert Mg^{2+} -sparing effects [345, 346]. Their beneficial effect on renal Mg^{2+} excretion, serum Mg^{2+} levels, and clinical symptoms is well documented in hereditary Mg^{2+} -wasting diseases [347].

Low-Renin Hypertension with Hypokalemia

Inherited Forms of Primary Aldosteronism

Epidemiological studies have shown that plasma aldosterone levels, renin levels, and the aldosterone to renin ratio (ARR) all correlate with increased blood pressure and the incidence of hypertension in the general population [348, 349, 350]. Autonomous aldosterone production from the adrenal cortex leads to primary aldosteronism, the most frequent form of secondary hypertension. The prevalence of primary aldosteronism among hypertensive patients is ~10 % [351], but as high as 20 % in patients with resistant hypertension [352]. Primary aldosteronism is associated with a suppressed renin–angiotensin

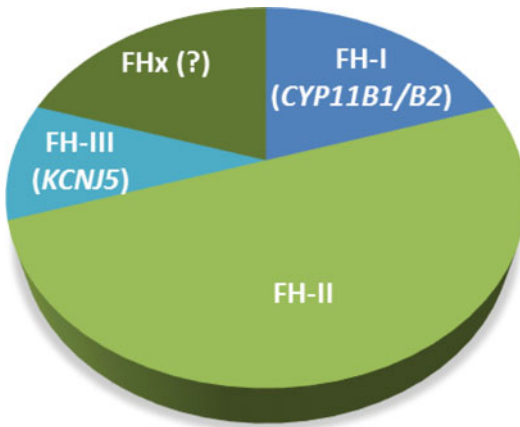


Fig. 4 Germline mutations in familial forms of primary aldosteronism. Three familial forms of PA have been described. FH-I is associated with a chimeric *CYP11B1/11B2* gene and FH-III with germline mutations in *KCNJ5*. FH-II is probably the most common form but without genetic abnormality identified so far. Other Mendelian forms of familial hyperaldosteronism (FHx) may also exist

system and often to hypokalemia. The two principal forms are unilateral aldosterone-producing adenoma (APA) and bilateral adrenal hyperplasia (BAH), also known as idiopathic hyperaldosteronism.

Only a minority (1–5 %) of primary aldosteronism cases are inherited familial forms. Three forms with Mendelian inheritance have been described: familial hyperaldosteronism type I (FH-I), type II (FH-II), and type III (FH-III) (Fig. 4).

Familial Hyperaldosteronism Type I

Familial hyperaldosteronism type I (FH-I, OMIM #103900), also called glucocorticoid-remediable aldosteronism (GRA) and dexamethasone-suppressible hyperaldosteronism (DSH), is a rare autosomal dominant form of primary aldosteronism. It was first individualized by Sutherland and coworkers [353] in a family with one father and in son and since then has been reported in less than 100 unrelated cases.

Clinical Features

The clinical and biochemical characteristics of the affected patients are variable, with a wide spectrum of presentations even within the same

family [354]. Affected individuals are usually hypertensive in the youth and demonstrate rapidly a severe form of hypertension, despite the fact that few families with a moderate phenotype have been described [355]. Investigating its prevalence in the pediatric population, Aglony and coworkers found four children with positive genetic testing among 130 hypertensive children [356]. The study of 21 first-degree relatives demonstrated a high variability in the clinical and biochemical characteristics of the affected patients.

In the hypertensive adult population, FH-I accounts for 0.5 % to 1.0 % of PA and occurs equally among women and men [357, 358].

Since the disease is transmitted as an autosomal dominant trait with a high penetrance, there is often a strong family history of hypertension and/or stroke [359]. A specific feature of the disease is the aldosterone hyperresponsiveness to maneuvers stimulating or inhibiting the pituitary adrenal axis [360]. Acute or chronic administration of ACTH induces a strong increase in plasma aldosterone level, whereas it has little or small effect on patients with other forms of primary aldosteronism. Conversely, aldosterone is suppressed by the administration of glucocorticoids, the acute dexamethasone suppression test being recognized as a diagnostic test for the pathology [361, 362]. The second specific feature is the abundant urinary production of hybrid steroids, 18-hydroxycortisol and 18-oxocortisol [363].

Genetics

In 1992, Lifton and colleagues [364] showed that FH-I was linked to an abnormal aldosterone synthase gene. They studied a large affected kindred and found a gene duplication arising from an unequal crossing over, resulting in a fusion of the 11 β -hydroxylase (*CYP11B1*) promoter with the coding sequence of the aldosterone synthase (*CYP11B2*) gene. In all families reported so far, the chimeric gene derives from unequal homologous recombination between intron 1 and intron 4 of the *CYP11B1* and *CYP11B2* genes, respectively. This recombination takes place always upstream of exon 5, since this exon contains two residues that differ between the two homologous enzymes [365] and that are critical to confer the

aldosterone synthase specificity. The chimeric gene encodes a protein that can hydroxylate and oxidate cortisol (the steroid substrate present in the zona fasciculata) in the 18-position. This gene is under the control of the 11 β -hydroxylase gene regulatory region, in which expression is under ACTH control and can be downregulated by exogenous glucocorticoid administration [366]. Therefore, aldosterone hypersecretion seems to mainly derive from the zona fasciculata. The genetic screening for this condition is easy to perform and is based on Southern blot or on a long-range PCR looking for the existence of a hybrid gene containing the 5' part of the *CYP11B1* gene and the 3' part of the *CYP11B2* gene [367]. Even if the condition is rare, clinicians should not hesitate to prescribe this genetic test since it is 100 % sensitive and specific in reference laboratories and since a positive finding strongly influences the medical care of the patient and possibly his family. An early-onset of hypertension (before 30 years of age) associated to biological features compatible with a hyperaldosteronism and a positive familial history of early hypertension and/or stroke before 50 years should encourage to perform this genetic test.

Therapy

FH-I should be treated with a glucocorticoid, preferably a synthetic glucocorticoid longer acting than hydrocortisone. In order to avoid complete suppression of the circadian regulation of cortisol and the development of iatrogenic Cushing's syndrome, the lowest possible dose of glucocorticoids which normalize blood pressure and serum potassium should be used [368]. The administration of dexamethasone at low doses (0.125–0.250 mg/day) only partially suppresses ACTH secretion and is able to correct hypertension for several years, as demonstrated by the maintenance of normal echocardiographic parameters [369]. A complementary treatment based on amiloride or spironolactone at low dose as well as other classical antihypertensive agents is often required. Eplerenone should be used in children to avoid glucocorticoid effects on growth or antiandrogenic effects of spironolactone [368].

Familial Hyperaldosteronism Type II (FH-II)

The Australian group led by R Gordon and M Stowasser described another form of familial primary aldosteronism, called type II (FH-II), not caused by the presence of the chimeric *CYP11B1/CYP11B2* gene [370]. It was initially detected in few families with about 30 % of the affected patients presenting an aldosterone-producing tumor [371]. The clinical and biological characteristics of these patients do not differ from those with sporadic primary aldosteronism, except that the trait seems inherited according to an autosomal dominant transmission with partial penetrance [372]. FH-II patients display a family history of primary aldosteronism, and within the same family, different subtypes may be present (aldosterone-producing adenoma and bilateral adrenal hyperplasia) [373, 374]. The screening of the entire genome in a large family with FH-II showed linkage with chromosome 7p22 [375]. In addition to two Australian kindreds, two other Italian families with FH-II were found to be linked to this locus [376], but no causal gene has been identified so far [377, 378].

The prevalence of FH-II has been estimated from 1 % to 6 % of the adult population suffering from primary aldosteronism [358, 373, 379]. Taking into account the frequency of sporadic primary aldosteronism in the adult hypertensive population, it might be possible that some so-called FH2 families reflect the coincidental aggregation of two individuals with a sporadic form of primary aldosteronism.

Familial Hyperaldosteronism Type III (FH-III)

In 2008, Geller and coworkers described a new familial form of primary aldosteronism in a father and two daughters with early-onset (between 4 and 7 years of age) severe resistant hypertension and hypokalemia [380]. Patients exhibited marked hyperaldosteronism and very high levels of the hybrid steroids 18-oxocortisol and 18-hydroxycortisol but no suppression of aldosterone production by dexamethasone treatment. All three individuals presented massive bilateral adrenal hyperplasia, confirmed by subsequent

pathological examination, requiring bilateral adrenalectomy to control blood pressure.

Recently, the genetic cause of FH-III has been attributed to a peculiar missense variant (p. Thr158Ala) in the *KCNJ5* gene, coding for the G protein-activated inward rectifier potassium channel GIRK4 [381]. This mutation is located near the GYG motif which confers GIRK4 K⁺ selectivity. Functional studies have demonstrated that the mutation leads to sodium influx into the cell and cell membrane depolarization. GIRK4_Thr158Ala-transduced adrenocortical cells present a loss in K⁺ selectivity, greater influx of Na⁺ into the cytoplasm resulting in depolarization of the plasma membrane, and activation of voltage-dependent Ca²⁺ channels. Accumulation of intracellular Ca²⁺ leads to activation of the calcium signaling pathway resulting in increased transcription of *CYP11B2* coding for aldosterone synthase and increased aldosterone production [382].

Recent studies have described different germline *KCNJ5* mutations in families with FH-III which are related to the severity of disease. Patients carrying the germline mutations p.Gly151Arg, which are also found as recurrent somatic mutations in APA, p. Thr158Ala, and p.Ile157Ser, all presented a severe phenotype of PA and early-onset hypertension resistant to medical treatment [383, 384]. Conversely, affected members of three FH-III families carrying the *KCNJ5* p.Gly151Glu mutation and affected members from one family carrying the *KCNJ5* p.Tyr152Cys mutation exhibited a much milder phenotype that was similar to FH-II [385, 386]. Discordant phenotypes have been also described such as a sporadic patient with hyperaldosteronism carrying a de novo germline heterozygous p.Gly151Arg mutation, who had developed polyuria at 1.5 years of age and hypertension and hypokalemia by age 4 years [387]. Thereafter, hyperaldosteronism was successfully treated for seven years with spironolactone without visible adrenal enlargement.

Pseudohyperaldosteronisms

Liddle Syndrome

In 1963, Liddle and colleagues described a family with hypertension and an abnormality of Na⁺

reabsorption at the level of the renal distal tubule which simulated primary aldosteronism but had negligible basal and stimulated aldosterone secretion [388] (OMIM #177200). Blood pressure and hypokalemia were not influenced by spironolactone treatment, but triamterene, a specific inhibitor of the distal renal epithelial Na⁺ channel, corrected these abnormalities. The authors proposed that the primary abnormality was a constitutive activation of the epithelial Na⁺ channel. Some 30 years later, this hypothesis was reinvestigated in the proband and in the originally described pedigree. The index case developed renal failure and renal transplantation corrected the aldosterone and renin responses to salt restriction. These features demonstrated the involvement of the kidney in the disease [389], making the epithelial amiloride-sensitive Na⁺ channel (ENaC) located in the CCD an attractive candidate gene for Liddle syndrome.

Genetics

Analyzing the original Liddle's pedigree, Dr Lifton and his group [390] showed complete linkage of the gene encoding the β -subunit of ENaC, located at chromosome 16p13-12. In this pedigree and in other unrelated kindreds, a premature stop codon, a frameshift mutation, and other deleterious mutations were found, all located in the last exon of the *SCNN1B* gene encoding the intracellular carboxy-terminal domain of the β -subunit. These mutations were showed to be gain-of-function mutations, with an increased amiloride-sensitive Na⁺ current after transfection of the corresponding mutant subunits together with α - and γ -wild-type subunits. In a Portuguese family affected with this syndrome, we found a 32-base-pair deletion leading to a premature termination of the carboxy-end of the same subunit [391]. Investigation of the epithelial nasal sodium/chloride conductance, as an alternative to the kidney, showed the presence of an increased amiloride-sensitive conductance in the three affected boys but not in their unaffected sister [392]. Other point mutations affecting the same region of the *SCNN1G* gene coding for the γ -subunit of ENaC have also been found to cause Liddle syndrome [393]. Interestingly, no mutation

of α -ENaC has been associated with Liddle syndrome so far. To date, more than 20 mutations which are located in the exon 13 of *SCNN1B* or *SCNN1G*, which cluster in very short segments of the C termini of either the β - or γ -subunit, have been identified [394].

Pathophysiology

ENaC is assembled as a trimer of three subunits, α , β , and γ which act together to confer its low Na^+ conductance and its high selectivity for Na^+ and amiloride [395]. The stoichiometry of the channel can be deduced with, from the crystal structure of a chicken, acid-sensing ion channel which belongs to the same family [396]. It is a heterotrimeric protein composed of one of each α , β , and γ subunit. ENaC is located in the apical membrane of epithelial cells and constitutes the rat-limiting step for sodium reabsorption, especially in the kidney, colon, and lung. Through hormones, especially aldosterone, ENaC expression is finely tuned by salt regimen, thus allowing appropriate changes in sodium reabsorption [397].

Comprehensive studies have shown that the mechanism by which the truncation of the C terminus of the β - and γ -subunits alters the ENaC function corresponds to an alteration of a conserved motif (PPxxY) in the C terminus of all three subunits of ENaC [398, 399]. In normal conditions a specific interaction between this PY motif and cytosolic proteins (Nedd4 isoforms 1 and 2 and other related WW proteins) leads to ubiquitylation and then degradation of the part of the newly synthesized subunits [400]. Thus, cell surface expression of ENaC is in part controlled via ubiquitylation which in itself is regulated by aldosterone-induced proteins and glucocorticoid-induced kinase 1 [401]. Both truncation and point mutations of the C-terminal PY motif increased surface expression of the mutant proteins and thus increased the number of sodium channels in the apical membrane [402], favoring renal Na^+ absorption and hypertension (Fig. 5). This results in expanded plasma volume which in turn inhibits the renin–aldosterone secretion. The fact that only one heterozygous mutation of either the β - or γ -ENaC subunit is sufficient to lead to the

pathology is probably due in part to the multimeric arrangement of the channel.

Diagnosis

The recognition of Liddle syndrome is important because it is potentially cured by the administration of amiloride. It is a form of autosomal dominant pseudoaldosteronism, i.e., hypertension associated with hypokalemia, metabolic alkalosis, and suppression of plasma renin but with very low levels of aldosterone in plasma and/or urine. Affected individuals are diagnosed at a relatively young age, most often between the age of 10 and 30 years [403]. These features are clearly different from the more severe and recessively transmitted apparent mineralocorticoid excess. The peculiar sensitivity to amiloride and the possibility of genetic testing allow a sure and rapid diagnosis. The genetic screening is based on the sequence analysis of the last exon 13 of the *SCNN1B* and *SCNN1G* genes.

Therapy

It is interesting to consider that a specific drug therapy for Liddle syndrome was developed in 1967 as a potassium-sparing diuretic [404], a long time before ENaC was cloned and was demonstrated to be responsible for the disease. Due to its very potent inhibiting properties on ENaC, amiloride is very effective in Liddle syndrome at doses comprised between 10 and 20 mg/day. Surprisingly for such a chronic and often severe condition, a change in blood pressure and in the biological profile can be observed as soon as after 2–4 weeks of treatment [405]. The inefficacy of spironolactone is easily understandable when one considers that renin and aldosterone are completely suppressed.

Activating Mutation of the Mineralocorticoid Receptor

One unique family with a new monogenic form of hypertension, characterized by early-onset and severe hypertension associated with low plasma levels of renin and aldosterone and exacerbation in pregnancy, was reported by Geller and coworkers [406] (OMIM #605115). It is caused by an activating missense mutation (Ser810Leu)

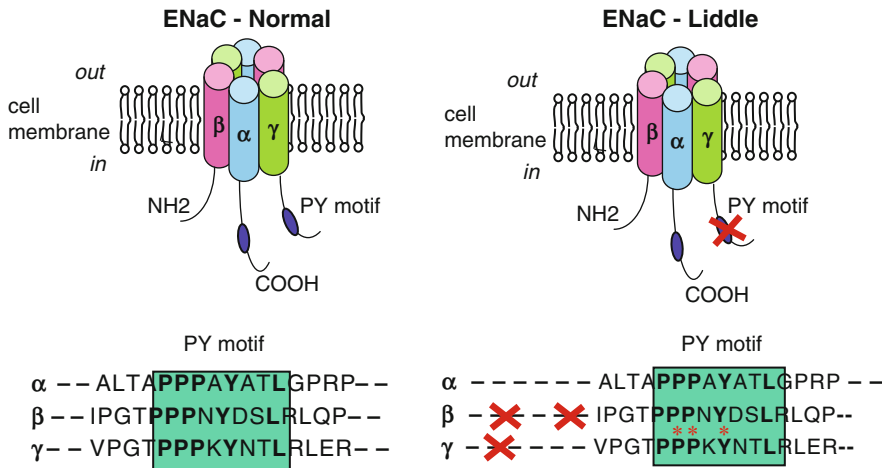


Fig. 5 Membrane topology of the epithelial Na⁺ channel ENaC. Mutations at the carboxy-terminal end of the β- and γ-subunits of the epithelial amiloride-sensitive Na channel (ENaC) in Liddle syndrome. A heterotrimeric model of the epithelial Na channel (ENaC) is represented. Each ENaC subunit (α, β, γ) has two transmembrane domains as well as a strongly conserved (PPxY) motif in their C-terminus portion. In normal conditions a specific interaction between this PY motif and cytosolic proteins (Nedd4 isoforms 1 and 2, and other

related WW proteins) leads to ubiquitylation and then degradation of the part of the newly synthesized subunits, thus affecting ENaC cell surface expression. All genetic variants causing Liddle syndrome disrupt directly or indirectly the C-terminal PY motif of either the β- or γ-ENaC subunits. As a consequence, they increased surface expression of the mutant proteins and thus increased the number of sodium channels in the apical membrane favoring renal Na⁺ absorption, plasma volume expansion, and hypertension

at the *NR3C2* gene that encodes the mineralocorticoid receptor (MR). This mutation is located within the hormone binding domain and results in a constitutive mineralocorticoid receptor activity and in an alteration of the receptor specificity. The receptor becomes abnormally activated by progesterone and other steroids lacking 21-hydroxyl groups which act normally as antagonists. In the family, all women bearing the mutation had severe pregnancy-induced hypertension with hypoaldosteronism which was likely caused by the massive increased production of progesterone during pregnancy.

Rafestin-Oblin and coworkers identified the endogenous steroids responsible for early-onset hypertension in men and nonpregnant women carrying the MR p.Ser810Leu mutation [407]. Indeed, cortisone and 11-dehydrocorticosterone, the main cortisol and corticosterone metabolites produced in the distal nephron by the action of 11β-hydroxysteroid dehydrogenase type 2, bind with high affinity to the mutant receptor, in sharp contrast to their low wild-type MR-binding

capacity. Increased binding is accompanied by MR-dependent transcriptional activation by both ligands in the presence of the p.Ser810Leu. Because the plasma concentration of cortisol in humans is about 30-fold higher than that of corticosterone, it is likely that cortisone triggers most of the phenotype in affected carriers, in the absence of pregnancy.

Importantly, spironolactone acts as an agonist on the mutated receptor instead of an antagonist. Thus its prescription in this very rare form of pseudohyperaldosteronism could be detrimental and is contraindicated. Amiloride may be effective in blocking MR-induced ENaC-mediated sodium reabsorption.

Apparent Mineralocorticoid Excess

The syndrome of apparent mineralocorticoid excess (AME) is a rare autosomal recessive form of hypertension (OMIM #218030) associated with suppressed renin and aldosterone levels, hypernatremia and hypokalemia, and metabolic alkalosis. It was first described by Dr New [408]

and Dr Ulick [409] in two subjects: one was a three-year-old Native American girl and the other one was a boy from Middle Eastern who had suffered a stroke at age 7 and was severely hypertensive. For both of them, clinical and biochemical evaluation failed to reveal overproduction of aldosterone or any other known steroid and established a new syndrome. AME is usually diagnosed within the first years of life and is characterized by a polyuria, polydipsia, failure to thrive together with severe hypertension associated with hyporeninism and hypoaldosteronism, a profound hypokalemia with alkalosis, and most often nephrocalcinosis [410].

This autosomal recessive syndrome is rare, with more than 100 cases being reported in the literature. The clinical and biochemical characteristics of AME, mimicking a very strong hyperaldosteronism, together with the frequent consanguinity between parents, make the diagnosis relatively easy. A few patients with a mild form of AME, also called AME type 2 (OMIM # 20 7765), have also been reported, with less caricatural hypertension and only mild abnormalities of cortisol metabolism [411, 412]. In a large Sardinian family, affected homozygous individuals were >30 years of age and had both mineralocorticoid hypertension and evidence of impaired metabolism of cortisol to cortisone, whereas heterozygous subjects were phenotypically normal with only subtle biochemical defects.

Pathophysiology and Genetics

The 11 β -hydroxysteroid dehydrogenase is a microsomal enzyme complex responsible for the interconversion of cortisol and cortisone. Two isoforms have been characterized: whereas the type I isoform (HSD11B1) is capable to have both the dehydrogenase and reductase activities, the type II isoform (HSD11B2) has only the 11 β -dehydrogenase activity and thus only catalyzes the cortisol to cortisone reaction (Lakshmi et al. 1985). Edwards and colleagues [413] showed that the HSD11B2 isoform is highly concentrated in the aldosterone responsive distal nephron and actually protects the mineralocorticoid receptor from a stimulation by cortisol whose plasma concentration is about 100-fold higher than aldosterone. Because of the defect in

the HSD11B2 isoform, AME patients are characterized by high values of the cortisol/cortisone ratio in plasma (F/E) and urine (THF/THE) and by arterial hypertension, mimicking a primary aldosteronism [410].

Dr P White's group first showed that AME is due to loss-of-function mutations in the HSD11B2 gene that encodes the 11 β -hydroxysteroid dehydrogenase type II isoform [414]. More than 50 mutations have been described. A genotype-phenotype relation exists: the more deleterious variants are, the more they affect the enzymatic activity in vitro and are associated with the higher increased urinary (THF+alloTHF)/THE ratios and thus with the more severe forms of hypertension [415–417]. HSD11B2 null mice provide a good model for the pathology [418].

Treatment

Two main strategies can be used to treat AME. The first one corresponds to the blockade of the mineralocorticoid receptor by spironolactone, thus acting as a competitive antagonist of endogenous cortisol. Daily doses of spironolactone between 2 and 10 mg/kg are usually sufficient to correct hypertension and increase natriuresis and renin levels [417]. The addition of thiazides can help to normalize blood pressure and lower hypercalciuria and nephrocalcinosis.

The second complementary strategy consists in using the administration of exogenous corticoids to suppress the endogenous secretion of cortisol. Doses up to 2 mg/day may be required. This strategy has also shown its efficiency on blood pressure, renin, and aldosterone but has little effect on urinary concentrations of the metabolites of cortisol, cortisone, and corticosterone [419]. Several reports have shown the curative effect of renal transplantation – with functional HSD11B2 activity – on the ionic anomalies, the cortisol/cortisone ratio, and blood pressure [420].

In addition to these two strategies, the use of nonspecific antihypertensive agents such as calcium antagonists may be necessary, due to the severity of hypertension [421]. Prospective long-term follow-up studies of children suffering from AME are missing to appreciate the long-term prognosis of the disease and treatment later in life.

Pseudohypoaldosteronisms

Pseudohypoaldosteronism Type II, Familial Hyperkalemic Hypertension

Pseudohypoaldosteronism type II (PHA2) (OMIM #145260), also known as familial hyperkalemic hypertension (FFHt) or Gordon syndrome, is an autosomal dominant form of volume-dependent hypertension characterized by hyperkalemia and hyperchloremic acidosis despite normal renal function [422].

Since the first description of the disease by Paver and Pauline in 1964, more than 100 other cases and families have been reported. Gordon and colleagues reported their first case in 1970 and helped to demonstrate the existence of a unifying syndrome [422]. Elective sensitivity to thiazide diuretics is a hallmark of the disease that is the mirror image of Gitelman syndrome. There is a strong variability, in terms of age at diagnosis, which may vary from the first few weeks of life until late in adulthood in sporadic and familial cases. Usually, the biochemical abnormalities precede the increase in blood pressure [423]. The phenotypic variability of FFHt is in part related to its genetic heterogeneity.

Genetics

Up to now, four genes have been implicated in the disease (Fig. 6). The first two, *WNK1* and *WNK4*, were identified through a classical approach of genome-wide linkage analysis [424, 425]. Both genes are members of a particular family of serine–threonine kinases, called with-no-lysine (K) kinase [426]. Disease-causing mutations in the *WNK1* gene are large deletions in the first intron which lead to increased gene expression of the active long isoform at the distal convoluted tubule, as demonstrated in the corresponding mouse model [427]. Linkage studies combined with whole exome sequencing in large families have recently identified two additional genes responsible for the disease, kelch-like family member 3 (*KLHL3*) and cullin 3 (*CUL3*) [428, 429]. The corresponding proteins belong to a ubiquitin-protein ligase complex. Genetic variants in *WNK4* will eventually be shown as

disrupting the relation between the *WNK4* acidic motif and the substrate adaptor *KLHL3*, and thus preventing the intrarenal degradation of the protein [430]. Disease-causing *KLHL3* variants are either recessive or dominant and mainly missense variants clustered in the kelch motifs of the protein. Conversely, all *CUL3* variants causing FFHt are leading to the elective abnormal splicing of exon 9, leading to the in-frame loss of 57 residues in the middle of the protein. Most of them occur de novo and few families with a vertical autosomal dominant transmission have been found. The severity of the disease associated with *CUL3* variants probably explains this selection pressure.

Pathophysiology

The identification of four genes responsible for PHA2 has allowed the discovery of a new cellular pathway regulating ionic reabsorption in the distal nephron and blood pressure regulation [431–433]. Up to now, all the in vitro and in vivo studies converge toward an increased abundance of *WNK4* in the kidney in case of disease-causing variants at the *WNK4*, *KLHL3*, and *CUL3* genes [434], whereas an increased abundance of the long kinase-active *WNK1* isoform in the distal nephron is the consequence of the large intron 1 deletions in *WNK1* [435]. In either cases, this increased abundance of *WNK4*/*WNK1* seems to mainly activate the NaCl cotransporter and cause hypervolemia, hyperkalemia, and acidosis together with an increase in blood pressure (Fig. 7).

WNK4 has been found to be a major player in regulating several renal ion transporters and channels. In vitro experiments showed that it inhibits *NCCT* activity in *Xenopus* oocytes by decreasing its surface expression and that mutated *WNK4* lost this ability to regulate *NCCT* (Wilson et al. 2003). It could also inhibit the activity of the renal apical K^+ channel ROMK, the basolateral isoform of the $Na^+K^+2Cl^-$ cotransporter (NKCC1), and the apical Cl^-/HCO_3^- exchanger CFEX and TRPV4 [431], again by decreasing their surface expression. Finally, *WNK4* was shown to stimulate the paracellular chloride transport, via phosphorylation of members of the claudin family that encode

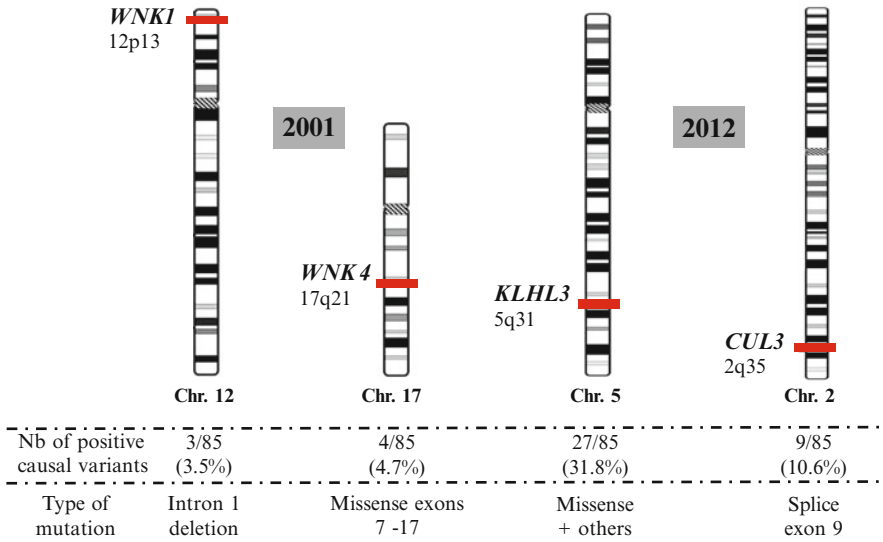


Fig. 6 Genetic heterogeneity of pseudohypoaldosteronism type II. Up to now, four genes have been identified as responsible for PHA2. The *WNK1* and *WNK4* genes at chr12 and chr17, respectively, were identified in 2001. The *KLHL3* and *CUL3* genes were identified in 2012. The number of positive causal variants at each

gene is indicated for the PHA2 cohort collected at the Department of Genetics in the Hôpital Européen Georges Pompidou in Paris, France. Further genetic heterogeneity probably exists since disease-causing genetic variants at these four genes explain roughly 70 % of our cases (Jeunemaitre, personal experience from the Paris Center)

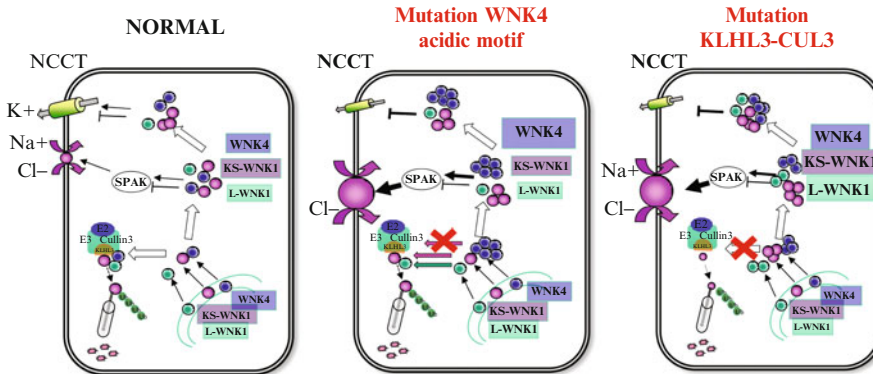


Fig. 7 Model to explain NCCT activation in case of PHA2-causing mutations at the *WNK4* or *KLHL3-CUL3* genes. The *WNK1* (L-WNK1 and KS-WNK1) and *WNK4* kinases are expressed in the distal convoluted tubule. The regulation of their activity is in part associated to the regulation of their intracellular abundance through their degradation by the proteasome, following ubiquitination through the E3 ubiquitin ligase complex containing *KLHL3* and *Cullin3*. *KLHL3* is a substrate adaptor for *WNK1* and *WNK4* through the degron acidic motif of these kinases. In case of variant at this *WNK4*

motif, the decreased interaction with *KLHL3* leads to an increased abundance and activity of this kinase, especially on the *SPAK* kinase and the *Na*–*Cl* cotransporter *NCCT*. Disease-causing variants at the *KLHL3* and *CUL3* genes have the same consequences on *WNK4* abundance but should also affect *WNK1* isoform abundance and activity. In either cases, this increased abundance of *WNK4*/*WNK1* seem to mainly activate the *NaCl* cotransporter and cause hypovolemia, hyperkalemia, and acidosis together with an increase in blood pressure

tight junction proteins. Transgenic mouse models showed that the main effect of the *WNK4* PHA2 mutations is an increased NCCT activity associated to a marked hyperplasia of the DCT [436]. *WNK4* modulates the NaCl reabsorption and K^+ secretion in response to aldosterone secretion. *WNK4* activity is also modulated by Ang II, itself stimulated by circumstances such as dehydration and hypovolemia, thus maximizing renal salt reabsorption without concomitantly increasing K^+ secretion [437].

PHA2-causing *WNK4* mutations cluster mainly in a conserved acidic domain, highly conserved between *WNK* kinases [425]. Ohta et al. mapped the *WNK* interaction site to a region containing a motif of ten amino acids "EPEEPEADQH" [438]. The recent report of the crystal structure of the *KLHL3* kelch domain in complex with the *WNK4* acidic motif revealed close polar contacts between several residues at this motif and other conserved residues at the surface of the kelch domain beta-propeller [430]. Thus, mutations in this degron motif abolish the interaction of *WNK4* with the ubiquitin ligase complex, thereby preventing its ubiquitination and proteasomal degradation (434 for review). Indeed, *WNK4* knock-in mice (*WNK4*^{+/*D561A*}) have markedly increased *WNK4* levels in the kidney [439].

The role of *WNK1* is more complex to analyze since multiple isoforms are produced from the *WNK1* gene due to the existence of three promoters, two polyadenylation sites, and several alternatively spliced exons [440]. The short kidney-specific isoform (KS-*WNK1*) lacks any kinase activity and is produced at a high level, exclusively in the DCT. The full-length (long) isoform (L-*WNK1*) is produced ubiquitously and at a low level all along the nephron. In vitro, L-*WNK1* and KS-*WNK1* have been shown to interact together and with other partners such as *WNK4*, the serum-glucocorticoid kinase 1 (SGK1), and ENaC. Most of the in vitro studies on L-*WNK1* have been hampered by the fact that they used rat L-*WNK1* cDNA with a missense mutation in its C-terminal portion [435]. Using an intact human L-*WNK1* cDNA, L-*WNK1* was shown to be a powerful activator of NCCT in both

Xenopus oocytes and HEK293 cells. It is supposed that the ratio between L and KS-*WNK1* is probably important in the distal nephron, adding a supplementary level of fine regulation of the ionic transport [432].

The role of intracellular chloride concentrations in the *WNK* kinase activity has been suspected since its discovery [441] and has recently been highlighted. L-*WNK1* is a chloride-sensitive kinase activated by a low Cl concentration [442]. Similarly, *WNK4* effect on NCCT activation was found to be modulated by intracellular chloride concentration when injected into *Xenopus* oocytes [443]. Thus, *WNK* kinases could exert differential effects on NCCT, depending on the intracellular chloride concentration.

Phenotypic Heterogeneity

It is not clear yet if the two genetic defects on *WNK1* and *WNK4* genes give an exact similar phenotype in terms of biochemical and clinical severity, because of the very low number of families detected up to now. The only biochemical difference that has been shown concerns calcium excretion. PHA2 families with *WNK4* mutation have been reported as having hypercalciuria, whereas normocalciuria was observed in the only *WNK1*-linked PHA2 family analyzed for this parameter [444]. In the *WNK4* pedigree analyzed by Farfel's group [445], affected members had hypercalciuria with normomagnesemia and decreased bone mineral density.

Patients with heterozygous *KLHL3* mutations have probably the milder phenotype both in terms of biochemical anomalies and arterial hypertension [428, 429]. Conversely, patients with the peculiar *CUL3* splice mutations leading to abnormal splicing of exon 19 have the most severe form of the disease, affecting children as soon as the first months and years of age [428].

Therapy

Low-salt regimen can be in part effective as also evidenced in other forms of volume-dependent hypertension [446]. Low-salt diet can be sufficient, especially in childhood, since blood pressure might be normal and chronic hyperkalemia is usually very well tolerated. Thiazide diuretics are

the treatment of choice since they interfere with the mechanism of the disease. Low doses of hydrochlorothiazide (i.e., 12.5–25 mg) are very efficient to correct both the biochemical abnormalities and the blood pressure in a few weeks. Thiazide diuretics also correct the hypercalciuria that is observed in WNK4-linked PHA2 [445]. Furosemide is also effective but is less logical since it increases the hypercalciuria and could enhance the risk of nephrolithiasis. From our experience, there is no loss of efficiency of thiazides along the years. However, their theoretical metabolic side effects are an argument to develop new antihypertensive agents controlling the new WNK pathway [447].

Pseudohypoaldosteronism Type I

Pseudohypoaldosteronism type I (PHA1, OMIM#177735, OMIM #264350) is a rare form of mineralocorticoid resistance characterized by neonatal renal salt wasting, failure to thrive, and dehydration. It is associated with hyponatremia, hyperkalemia, and metabolic acidosis, despite extremely high values of plasma renin and aldosterone [448]. It was first reported by Cheek and Perry, who described a male infant with severe salt wasting in the absence of any renal or adrenal defect [449]. There exist two different clinical forms of PHA1: (i) a renal form, in which mineralocorticoid resistance is restricted to the kidney, and (ii) a generalized form, where mineralocorticoid resistance is systemic and salt loss occurs in multiple organs [450].

Clinical and Biochemical Features

Renal PHA1. Renal PHA1 (MIM#177735, also called autosomal dominant PHA1) is a mild form and represents the most frequent form of the disease [451]. The prevalence, as estimated from recruitment through a national reference center for rare diseases (PHA1.NET, coordinator MC. Zennaro; MARHEA, <http://www.soc-nephrologie.org/marhea/>) is ~1 per 80,000 newborns. Clinical expression is variable: in general, patients show a salt-losing syndrome in the neonatal period, with weight loss, failure to thrive,

vomiting, and dehydration. Occasionally, patients may also present hypercalciuria. Biological findings are hyponatremia, hyperkalemia, and inappropriately high urinary sodium excretion. In contrast, urinary potassium excretion is low, with reduced fractional potassium excretion and transtubular potassium gradient [452, 453]. The diagnosis is confirmed by the presence of high plasma and urinary aldosterone and high plasma renin levels. Symptoms of renal PHA1 usually improve in early childhood. The mechanisms which restore sodium homeostasis in these patients are not clear; most likely, kidney maturation, access to dietary salt, compensatory increase in proximal sodium reabsorption, and the upregulation of the mineralocorticoid axis all play a role to compensate for the distal salt loss. A recent case–control study, comparing 39 adult patients with renal PHA1 carriers of *NR3C2* mutations with sex- and age-paired noncarriers, showed that adult PHA1 subjects present lifelong increased plasma renin and aldosterone levels together with increased salt appetite, with normal potassium levels and blood pressure [454]. Despite lifelong increase in plasma aldosterone and renin levels, no adverse cardiovascular outcome occurred in these patients, who rather show an improved diastolic left ventricular function.

Generalized PHA1. In contrast to the renal form, patients affected by generalized PHA1 (MIM #264350, also called autosomal recessive PHA1) have severe salt wasting from the kidney, the colon, sweat, and salivary glands [455]. Patients present in the neonatal period with severe dehydration, vomiting, and failure to thrive; acute polyhydramnios has been described [456], but is an exceptional finding (PHA1.NET, unpublished observation). The clinical picture may be complicated by cardiac dysrhythmias, collapse, shock, or cardiac arrest [457]. Severe hyperkalemia and high aldosterone and plasma renin levels orient the diagnosis that can be completed by a positive salivary or sweat test. Consistent with the systemic mineralocorticoid unresponsiveness, patients present elevated sweat and salivary Na^+ and Cl^- and absent nasal amiloride-sensitive Na^+ transport [450]. In addition to the renal phenotype, frequent respiratory tract illnesses have been

observed, characterized by persistent rhinorrhea of clear liquid, congestion, tachypnea, wheezing, frequent fever, and recurrent pulmonary infections, evoking cystic fibrosis, due to their reduced capacity to absorb liquid from airway surfaces [458]. Also, the high concentration of sweat salt may cause inflammation and damage in the eccrine structures, with cutaneous lesions similar to those appearing in miliaria rubra [457, 459]. This form of PHA1 is more severe and the prognosis is poor: no remission has been reported and patients suffer from recurrent life-threatening episodes of salt loss. Early diagnosis within the first week of life is critical to survive in generalized PHA1 [460].

Secondary PHA1. Transient pseudohypoaldosteronism has been observed in infants less than 7 months of age suffering from urinary tract malformations or urinary tract infections or both [461–463]. Any kind of congenital urinary tract obstruction (ureterohydronephrosis, ureterocele, ureteropelvic junction obstruction, posterior urethral valves) may lead to secondary PHA1. Physiopathological mechanisms responsible for transient PHA1 are poorly understood, but the gravity of the salt wasting is inversely correlated with age, supporting a role for tubular immaturity [460]. In these patients, medical or surgical care of the primary disease reestablishes the normal response to aldosterone although high-salt intake is recommended in the acute phase with progressive tapering. The possibility that a transient tubular mineralocorticoid resistance can arise in infants with urinary tract malformations or urinary tract infections strongly supports an indication for renal ultrasonography and urine cultures in all children presenting with salt wasting and hyperkalemia [461]. Secondary PHA1 may also develop in the adult following resection of the ileum and colon [464] or in patients with kidney transplantation treated with immunosuppressants (tacrolimus, cyclosporine), probably due to impairment of the mineralocorticoid receptor transcriptional activity [465].

Pathophysiology and Genetics

Aldosterone plays a key role in electrolyte balance and blood pressure regulation. It confers the main

hormonal regulation of sodium, potassium, and hydrogen balance in the aldosterone-sensitive distal nephron, including the late distal convoluted tubule, the connecting tubule, and the collecting duct, through its interaction with the mineralocorticoid receptor [466] (Fig. 8). The MR belongs to the nuclear receptor superfamily and acts as a ligand-activated transcription factor regulating expression of a coordinate set of genes ultimately eliciting physiologic aldosterone responses [467]. In the collecting duct, aldosterone stimulates activity of several proteins implicated in transepithelial sodium transport, including ENaC and $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ [467].

Since the first description of PHA1, a defect of the tubular response to aldosterone had been suggested as the underlying cause. After the characterization of aldosterone binding sites in mononuclear leukocytes, Armanini et al. confirmed this hypothesis by showing that ^3H -aldosterone binding was absent or very low in mononuclear leukocytes of affected patients [468]. In a subsequent study of eight families, a dual pattern of inheritance was observed that correlated with receptor binding abnormalities. In some families an autosomal recessive inheritance of the disease was observed, and binding studies and aldosterone levels were normal in both parents. In contrast, some families presented with an autosomal dominant inheritance, and low receptor number and elevated plasma aldosterone were always found in one of the parents [469]. The main differences between the renal and generalized forms of PHA1 are summarized in Table 7.

Subsequently it was shown that the two clinical forms of PHA1 were caused by different genetic defects. Inactivating mutations in the *NR3C2* gene coding for the MR are found in renal PHA1 [470–472] (Fig. 9). The human MR coding gene *NR3C2* spans over ~450 kB on chromosome 4p31.23 and is composed of ten exons [473]. Patients are heterozygous carriers of genetic variants which can be found in both patients with familial autosomal dominant PHA1 and patients with a sporadic renal presentation [472]. In the latter group, only one third are de novo mutations, implying that carriers develop clinically evident disease only in a small

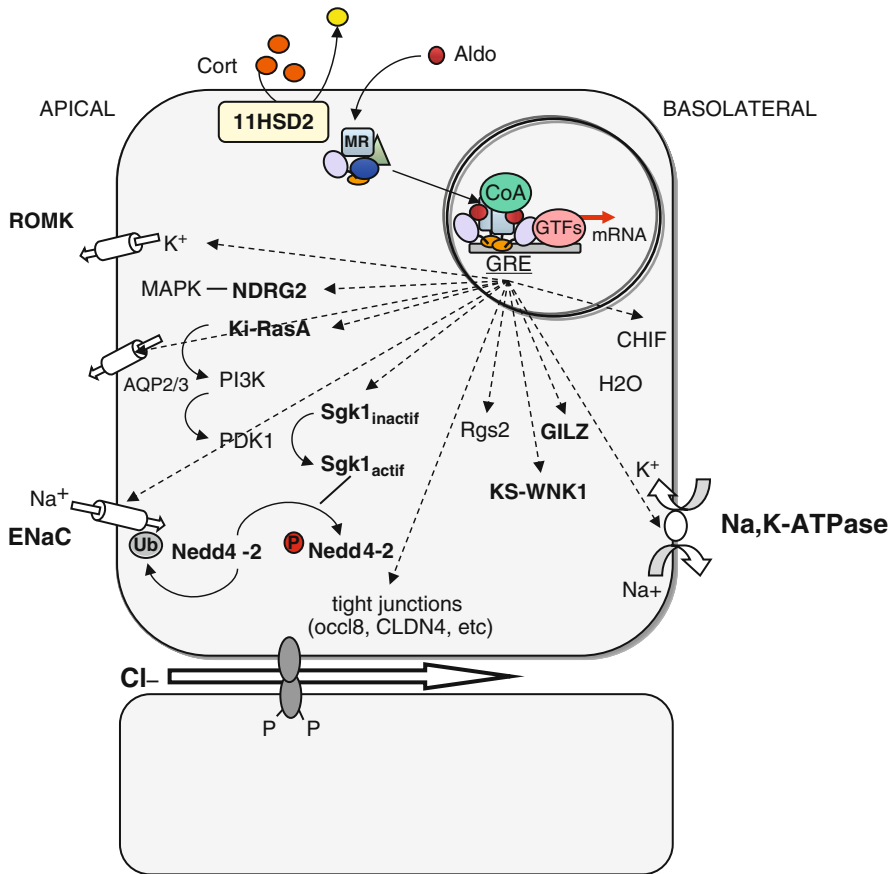


Fig. 8 Molecular basis of pseudohypoaldosteronism type I. Aldosterone binds to its intracellular receptor, the mineralocorticoid receptor (MR). Hormone binding induces dissociation of MR-associated proteins and translocation of the aldosterone-receptor complex into the nucleus, where it binds to specific DNA sequences in regulatory regions of hormone-responsive genes. These genes code for proteins involved in transepithelial sodium

transport (the epithelial sodium channel, ENaC; the sodium-potassium pump Na-K-ATPase), for regulatory proteins (serum- and glucocorticoid-regulated kinase 1 (sgk1), channel-inducing factor (CHIF), the proto-oncogene K-Ras2, N-myc downregulated gene 2 (NDRG2)), and others. Aldo, aldosterone; apical and basolateral indicate the two poles of the epithelial cell

proportion of kindreds. Although the exact causes for the variable phenotypic expression of renal PHA1 are unknown, intercurrent events, such as infections, vomiting, and diarrhea, may precipitate neonatal salt loss and dehydration in individuals at risk. Also, naturally occurring hypomorphic or hyperfunctioning alleles of other genes, coding for proteins involved in distal sodium reabsorption, may aggravate or attenuate the phenotype.

After the first description of *NR3C2* mutations in four dominant and one sporadic case of PHA1

[470], more than fifty different causal genetic variants have been reported in patients with renal PHA1 [448]. Disease-causing variants are located in all exons of the *NR3C2* gene, affecting all functional domains of the MR protein. However, only frameshift, splice site, and nonsense mutations were reported in exon 2, suggesting that missense mutations affecting the N-terminal domain do not sufficiently affect MR function to become clinically apparent.

The pathogenic mechanism of PHA1 in patients with heterozygous MR mutations

Table 7 Distinctive clinical and biological features of renal and generalized PHA1

Feature	Renal PHA1	Generalized PHA1
OMIM #	177735	264350
Inheritance	AD	AR
Age of onset	Neonatal	Neonatal
Phenotype	Mild	Severe
Failure to thrive	Present	Present
Vomiting	Present	Present
Dehydration episodes	Mild	Severe
Cardiac dysrhythmias, collapse, shock, or cardiac arrest	No	Sometimes
Respiratory tract illnesses	No	Frequent
Eczematoid rash of the skin	No	Sometimes
Hyperkalemic metabolic acidosis	Present	Present
Serum Na ⁺	↓	↓
Serum K ⁺	↑	↑
Urinary Na ⁺ excretion	↑	↑
Urinary Ka ⁺ excretion	↓	↓
Plasma aldosterone	↑	↑
Plasma renin	↑	↑
Urinary aldosterone	↑	↑
Sweat sodium	N	↑
Salivary sodium	N	↑
Type of mutation	Loss of function	Loss of function
Gene locus	4q31.1	12p13, 16p13-p12, 16p13-p12
Genes	NR3C2	SCNN1A, SCNN1B, SCNN1G
Proteins	MR, mineralocorticoid receptor	α-ENaC, β-ENaC, γ-ENaC, α-, β-, γ-subunits of the epithelial sodium channel

depends on the mutation. Although haploinsufficiency is sufficient to cause autosomal dominant PHA1 [474, 475], mutated receptors may also exert dominant-negative effects on the wild-type receptor since MR regulate transcription by binding as receptor dimer to regulatory regions of target genes. In this case, effects of mutated receptors are strongly promoter-dependent and may differentially affect MR function in a gene-specific manner [476, 477]. Furthermore, the same mutation may induce complete functional loss of transcriptional activity on one promoter, while retaining a partial transcriptional activity on another gene [477]. Thus, not only the extent of functional reduction but also the specific qualitative loss of function, in terms of regulated gene expression, modulates the phenotype in PHA1 patients with *NR3C2* mutations.

The severe and generalized, recessive form of PHA1 is caused by loss-of-function mutations at the genes coding for the subunits of the amiloride-sensitive sodium channel ENaC (*SCNN1A*; *SCNN1B*; *SCNN1G*). ENaC is a heteromultimeric protein composed of three subunits, α, β, and γ, which form the channels expressed on the cell surface (see review by [478]). The α-subunit of ENaC is encoded by *SCNN1A* located on chromosome 12p13.3, whereas *SCNN1B* (encoding β-ENaC) and *SCNN1G* (encoding γ-ENaC) are located within 400 kb on chromosome 16p12.2-p13.11. Loss-of-function mutations have been found in affected patients being either homozygous – in consanguineous families – or composite heterozygous [479, 480]. They include missense, nonsense, deletion, insertion, and splice site junction mutations leading to abnormal mRNA splicing. PHA1-causing variants appear in all ENaC subunits, but are more frequent in the α-subunit, consistent with its determinant role in channel function. Missense mutations are found in critically important domains of the protein and affect functions like intracellular trafficking of the channel, channel gating, or the ion selectivity filter [481].

Genotype–phenotype correlations are not well established in systemic PHA1. A recent study has explored the long-term consequences of different

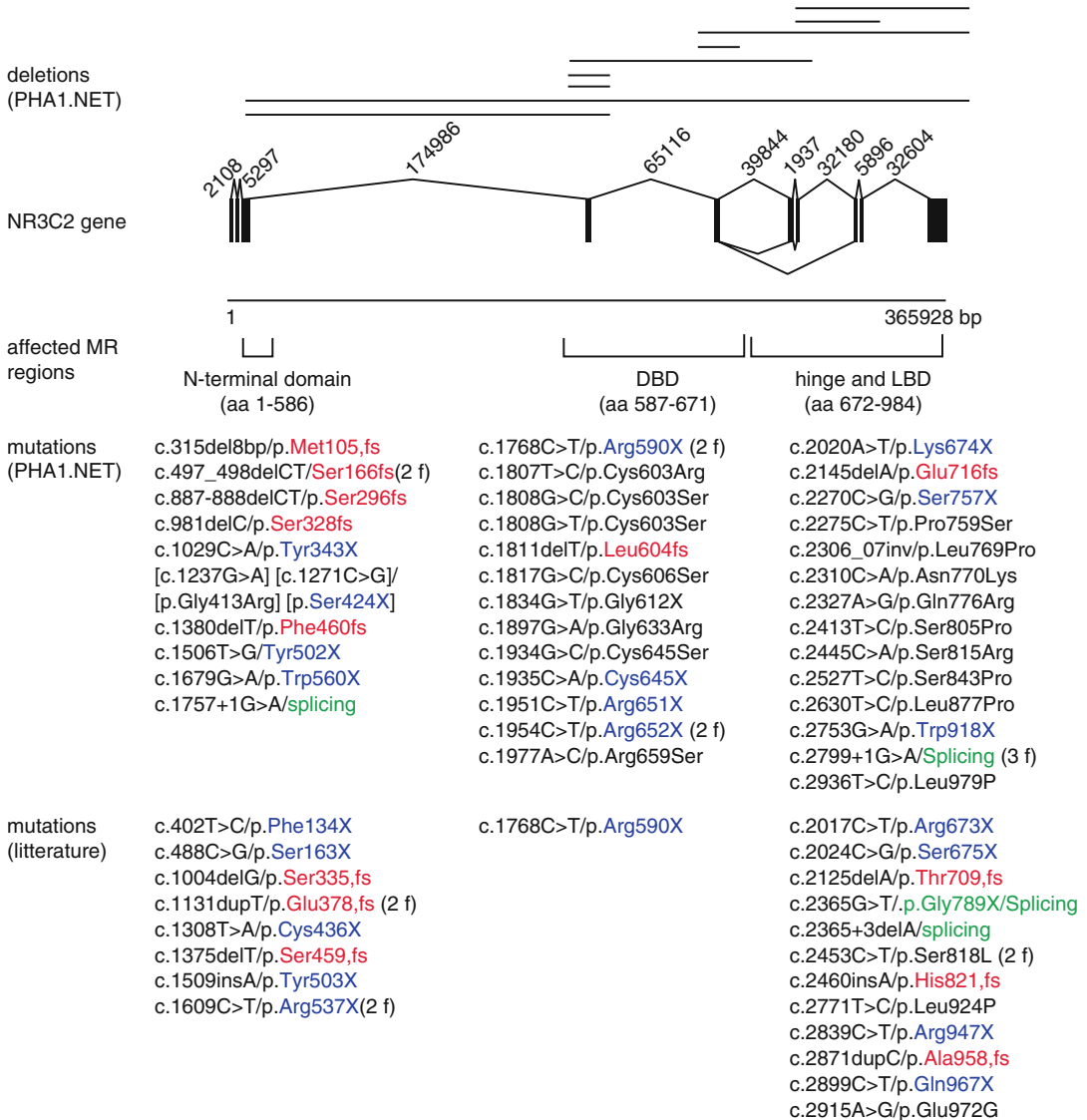


Fig. 9 *NR3C2* mutations in renal pseudohypoaldosteronism type I. The *NR3C2* gene is represented with its intron/exon structure (exons are represented by boxes or black lines, introns by spaces in between). Eight exons [2–9] code for the functional domains of the MR protein. *NR3C2* mutations identified in PHA1 are depicted on the

gene. The functional domains of the MR are indicated. DBD, DNA binding domain; LBD, ligand binding domain; (PHA.NET), unpublished mutations identified in the context of the clinical and research network PHA.NET (PI: M-C. Zennaro, Paris)

α -ENaC mutations on the renin–aldosterone system, growth, and pubertal development of PHA1 patients [482]. Three patients homozygous for nonsense and frameshift mutations in α -ENaC presented short stature, poor growth, and growth hormone tests compatible with the diagnosis of GH deficiency. In all patients, there was an

age-dependent normalization in the urinary Na/K ratios accompanied by an exaggerated renin–aldosterone system response probably contributing to age-dependent amelioration. In contrast, one patient compound heterozygous for a missense mutation and for a frameshift mutation of α -ENaC presented normal growth, normal

puberty, and decrease of renin–aldosterone axis activity with age. These results demonstrate distinct genotype–phenotype relationships in generalized PHA1 patients that depend on the degree of functional ENaC impairment. If undetected during the first week of life, multisystem PHA1 can lead to neonatal death; however, if detected, the patients may lead near normal lives on a lifelong high-salt diet [483].

Extensive genetic investigation of PHA1 patients in recent years has also shown that the disease comprises a continuum of phenotypically and/or biologically distinct entities that may challenge the current genetic classification of the disease. Indeed, investigation of heterozygote carriers of a α -ENaC p.Ser562Pro mutation, responsible for generalized PHA1 in homozygous patients, revealed a subclinical salt-losing phenotype with increased sweat sodium and chloride concentrations without additional hormonal or clinical manifestation [484]. Furthermore, recessively inherited ENaC mutations, associated with partial loss of channel function, may result in a mild phenotypic expression of PHA, with a salt-losing disorder in a premature infant, but only a biological phenotype in a sibling born at term [485]. We recently observed a case of recessive PHA1 with an extremely severe phenotype of dehydration and hyperkalemia, but without cutaneous or pulmonary phenotype, caused by MR mutations [486]. These cases broaden the spectrum of clinical phenotypes in renal PHA1 and support corresponding genetic screening for the disease in patients with isolated renal salt-losing syndromes and/or failure to thrive.

Treatment and Prognosis

Treatment of PHA1 consists in the replacement of salt loss and rehydration, as well as correction of hyperkalemia and acidosis in the acute phase of the disease. Since the main differential diagnosis is congenital adrenal hyperplasia or isolated deficiency in aldosterone synthase (CMOI and CMOII) [487], replacement therapy with fludrocortisone and hydrocortisone may be undertaken while confirming the diagnosis by hormonal measurements. Early postnatal hyperkalemia may sometimes complicate antenatal Bartter syndrome

(aBS, due to mutation in the potassium channel ROMK) [488]. Its association with hyponatremia and hyperreninemic hyperaldosteronism may erroneously suggest the diagnosis of PHA1. However, hyperkalemia appears usually very early and normalizes by the end of the first postnatal week, whereas PHA1 is characterized by permanent hyperkalemia. Other distinctive features of aBS patients are metabolic alkalosis as well as hypercalciuria and nephrocalcinosis. Also maternal hydramnios, present in aBS, is a rare event in generalized PHA1.

After the acute period, treatment consists in salt supplementation. The doses vary depending on the severity of the disease. Neonatal genetic diagnosis on cord blood may allow rapid diagnosis and management of the condition in affected offspring from PHA1 families with identified mutations. In renal and secondary PHA1, 3–20 mEq/kg/day of Na^+ given as NaCl and NaHCO_3^- are sufficient to compensate for the salt loss and are followed by rapid clinical and biochemical improvement. The expansion of extracellular volume results in increased tubular flow and delivery of sodium to the distal nephron, creating a favorable gradient for potassium secretion. Nevertheless, ion exchange resins are often associated to the treatment to normalize potassium levels. The amount of sodium required depends on the severity of the symptoms and is deduced from the normalization of plasma potassium concentration and plasma renin. Since renal PHA1 improves with age, treatment can be discontinued after a variable period of time in most patients, generally around age 18–24 months. Older children are generally asymptomatic on a normal salt intake and show a normal growth and psychomotor development, although they may evolve on the lower percentiles of the growth curve, despite adequate medical therapy [489].

In contrast to renal PHA1, generalized PHA1 represents a therapeutic challenge. No evidence-based treatment has been described, and therapeutic intervention is patient specific. Generally, high doses of sodium (between 20 and 50 mEq/kg/day) are used, together with ion exchange resins and dietary manipulations to reduce potassium levels. Corticoid treatment is sometimes associated and seems to provide some additional benefit.

Administration of indomethacin may be useful in occasional patients [490]. Symptomatic treatment is necessary for the respiratory tract illnesses and to correct the skin phenotype. Only few cases of generalized PHA1 followed up for several years or into adulthood have been described: treatment is necessary throughout life, consisting of salt supplementation (8–20 g NaCl/day) and ion exchange resins [460, 491]

References

- Bartter F, Pronove P, Gill Jr J, MacCardle R. Hyperplasia of the juxtaglomerular complex with hyperaldosteronism and hypokalemic alkalosis. A new syndrome. *Am J Med.* 1962;33:811–28.
- Jeck N, Schlingmann KP, Reinalter SC, Kömhoff M, Peters M, Waldeger S, Seyberth HW. Salt handling in the distal nephron: lessons learned from inherited human disorders. *Am J Physiol Regul Integr Comp Physiol.* 2005;288(4):R782–95.
- Greger R. Ion transport mechanisms in thick ascending limb of Henle's loop of mammalian nephron. *Physiol Rev.* 1985;65:760–97.
- Reilly RF, Ellison DH. Mammalian distal tubule: physiology, pathophysiology and molecular anatomy. *Physiol Rev.* 2000;80:277–313.
- Hoenderop JG, Bindels RJ. Epithelial Ca²⁺ and Mg²⁺ channels in health and disease. *J Am Soc Nephrol.* 2005;16:15–26.
- 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.* 1996;13:183–8.
- 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.* 1996;14:152–6.
- Birkenhager R, Otto E, Schurmann MJ, Vollmer M, Ruf EM, Maier-Lutz I, Beekmann F, Fekete A, Omran H, Feldmann D, Milford DV, Jeck N, Konrad M, Landau D, KnoersNV AC, Sudbrak R, Kispert A, Hildebrandt F. Mutation of BSND causes Bartter syndrome with sensorineural deafness and kidney failure. *Nat Genet.* 2001;29:310–4.
- 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.* 1997;17:171–8.
- Simon DB, Nelson-Williams C, Bia MJ, Ellison D, Karet FE, Molina AM, Vaara I, Iwata F, Cushner HM, Koolen M, Gainza FJ, Gitelman HJ, Lifton RP. Gitelman's variant of Bartter's syndrome, inherited hypokalemic alkalosis, is caused by mutations in the thiazide sensitive Na-Cl cotransporter. *Nat Genet.* 1996;12:24–30.
- Konrad M, Vollmer M, Lemmink HH, Van Den Heuvel LP, Jeck N, Vargas-Poussou R, Lakings A, Ruf R, Deschenes G, Antignac C, Guay-Woodford L, KnoersNV SHW, Feldmann D, Hildebrandt F. Mutations in the chloride channel gene CLCNKB as a cause of classic Bartter syndrome. *J Am Soc Nephrol.* 2000;11:1449–59.
- McCredie DA, Rotenberg E, Williams AL. Hypercalciuria in potassium-losing nephropathy: a variant of Bartter's syndrome. *Aust Paediatr J.* 1974;10(5):286–95.
- Proesmans W. Bartter syndrome and its neonatal variant. *Eur J Pediatr.* 1997;156(9):669–79.
- Vargas-Poussou R, Feldmann D, Vollmer M, Konrad M, Kelly L. Novel molecular variants of the Na-K-2Cl cotransporter gene are responsible for antenatal Bartter syndrome. *Am J Hum Genet.* 1998;62:1332–40.
- Seyberth HW, Koniger SJ, Rascher W, Kuhl PG, Schweer H. Role of prostaglandins in hyperprostaglandin E syndrome and in selected renal tubular disorders. *Pediatr Nephrol.* 1987;1:491–7.
- Kockerling A, Reinalter SC, Seyberth HW. Impaired response to furosemide in hyperprostaglandin E syndrome: evidence for a tubular defect in the loop of Henle. *J Pediatr.* 1996;129:519–28.
- 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.* 1997;6(1):17–26.
- Bettinelli A, Ciarmatori S, Cesareo L, Tedeschi S, Ruffa G, Appiani AC, Rosinini A, Crumieri G, Mercuri B, Sacco M, Leozappa G, Binda S, Ceconi M, Navone C, Curcio C, Syren ML, Casari G. Phenotypic variability in Bartter syndrome type I. *Pediatr Nephrol.* 2000;14:940–5.
- Nielsen S, Maunsbach AB, Ecelbarger CA, Knepper MA. Ultrastructural localization of Na-K-2Cl cotransporter in thick ascending limb and macula densa of rat kidney. *Am J Physiol Renal Physiol.* 1998;275:F885–93.
- Shankar SS, Brater DC. Loop diuretics: from the Na-K-2Cl transporter to clinical use. *Am J Physiol Renal Physiol.* 2003;284:F11–21.
- Adachi M, Asakura Y, Sato Y, Tajima T, Nakajima T, Yamamoto T, Fujieda K. Novel SLC12A1 (NKCC2) mutations in two families with Bartter syndrome type 1. *Endocr J.* 2007;54:1003–7.
- 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.* 1997;8(11):1706–11.
23. Castrop H, Schnermann JB. Isoforms of the renal Na-K-2Cl cotransporter NKCC2: expression and functional significance. *Am J Physiol Renal Physiol.* 2008;295(4):F859–66.
 24. Wang W, Sackin H, Giebisch G. Renal potassium channels and their regulation. *Annu Rev Physiol.* 1992;54:81–96.
 25. Nichols CG, Lopatin AN. Inward rectifier potassium channels. *Annu Rev Physiol.* 1997;59:171–91.
 26. Feldmann D, Alessandri JL, Deschênes G. Large deletion of the 5' end of the ROMK1 gene causes antenatal Bartter syndrome. *J Am Soc Nephrol.* 1998;9(12):2357–9.
 27. Flagg TP, Tate M, Merot J, Welling PA. A mutation linked with Bartter's syndrome locks Kir 1.1a (ROMK1) channels in a closed state. *J Gen Physiol.* 1999;114(5):685–700.
 28. Schulte U, Hahn H, Konrad M, Jeck N, Derst C, Wild K, Weidmann S, Ruppertsberg JP, Fakler B, Ludwig J. pH gating of ROMK (K(ir)1.1) channels: control by an Arg-Lys-Arg triad disrupted in antenatal Bartter syndrome. *Proc Natl Acad Sci U S A.* 1999;96(26):15298–303.
 29. Jeck N, Derst C, Wischmeyer E, Ott H, Weber S, Rudin A, Seyberth HW, Daut J, Karschin A, Konrad M. Functional heterogeneity of ROMK mutations linked to hyperprostaglandin E syndrome. *Kidney Int.* 2001;59:1803–11.
 30. Starremans PG, van der Kemp AW, Knoers NV, van den Heuvel LP, Bindels RJ. Implications of mutations in the human renal outer medullary potassium channel (ROMK2) identified in Bartter syndrome. *Pflugers Arch.* 2002;443(3):466–72.
 31. Peters M, Jeck N, Reinalter S, Leonhardt A, Tonshoff B, Klaus G, Konrad M, Seyberth HW. Clinical presentation of genetically defined patients with hypokalemic salt-losing tubulopathies. *Am J Med.* 2002;112:183–90.
 32. Madrigal G, Saborio P, Mora F, Rincon G, Guay-Woodford LM. Bartter syndrome in Costa Rica: a description of 20 cases. *Pediatr Nephrol.* 1997;11(3):296–301.
 33. Vaisbich MH, Fujimura MD, Koch VH. Bartter syndrome: benefits and side effects of long-term treatment. *Pediatr Nephrol.* 2004;19(8):858–63.
 34. Seyberth HW, Rascher W, Schweer H, Kühl PG, Mehls O, Schärer K. Congenital hypokalemia with hypercalciuria in preterm infants: a hyperprostaglandinuric tubular syndrome different from Bartter syndrome. *J Pediatr.* 1985;107(5):694–701.
 35. Shoemaker L, Welch TR, Bergstrom W, Abrams SA, Yergey AL, Vieira N. Calcium kinetics in the hyperprostaglandin E syndrome. *Pediatr Res.* 1993;33(1):92–6.
 36. Rodríguez-Soriano J, Vallo A, Aguirre M. Bone mineral density and bone turnover in patients with Bartter syndrome. *Pediatr Nephrol.* 2005;20(8):1120–5.
 37. Pressler CA, Heinzinger J, Jeck N, Waldegger P, Pechmann U, Reinalter S, Konrad M, Beetz R, Seyberth HW, Waldegger S. Late-onset manifestation of antenatal Bartter syndrome as a result of residual function of the mutated renal Na⁺-K⁺-2Cl⁻ co-transporter. *J Am Soc Nephrol.* 2006;17(8):2136–42.
 38. Schachter AD, Arbus GS, Alexander RJ, Balfé JW. Non-steroidal antiinflammatory drug-associated nephrotoxicity in Bartter syndrome. *Pediatr Nephrol.* 1998;12:775–7.
 39. Chaudhuri A, Salvatierra Jr O, Alexander SR, Sarwal MM. Option of pre-emptive nephrectomy and renal transplantation for Bartter's syndrome. *Pediatr Transplant.* 2006;10(2):266–70.
 40. Rudin A. Bartter's syndrome: a review of 28 patients followed for 10 years. *Acta Med Scand.* 1988;224:165–71.
 41. Reinalter SC, Grone HJ, Konrad M, Seyberth HW, Klaus G. Evaluation of long-term treatment with indomethacin in hereditary hypokalemic salt-losing tubulopathies. *J Pediatr.* 2001;139:398–406.
 42. Taugner R, Waldherr R, Seyberth HW, Erdös EG, Menard J, Schneider D. The juxtaglomerular apparatus in Bartter's syndrome and related tubulopathies. An immunocytochemical and electron microscopic study. *Virchows Arch A Pathol Anat Histopathol.* 1988;412(5):459–70.
 43. Okada M, Lertprasertsuke N, Tsutsumi Y. Quantitative estimation of rennin-containing cells in the juxtaglomerular apparatus in Bartter's and pseudo-Bartter's syndromes. *Pathol Int.* 2000;50:166–8.
 44. Finer G, Shalev H, Birk OS, Galron D, Jeck N, Sinaitreiman L, Landau D. Transient neonatal hyperkalemia in the antenatal (ROMK defective) Bartter syndrome. *J Pediatr.* 2003;142(3):318–23.
 45. Ji W, Foo JN, O'Roak BJ, Zhao H, Larson MG, Simon DB, Newton-Cheh C, State MW, Levy D, Lifton RP. Rare independent mutations in renal salt handling genes contribute to blood pressure variation. *Nat Genet.* 2008;40(5):592–9.
 46. Tobin MD, Tomaszewski M, Braund PS, Hajat C, Raleigh SM, Palmer TM, Caulfield M, Burton PR, Samani NJ. Common variants in genes underlying monogenic hypertension and hypotension and blood pressure in the general population. *Hypertension.* 2008;51(6):1658–64.
 47. Devuyt O. Salt wasting and blood pressure. *Nat Genet.* 2008;40(5):495–6.
 48. Welling PA. Rare mutations in renal sodium and potassium transporter genes exhibit impaired transport function. *Curr Opin Nephrol Hypertens.* 2014;23(1):1–8.
 49. Mourani CC, Sanjad SA, Akatcherian CY. Bartter syndrome in a neonate: early treatment with indomethacin. *Pediatr Nephrol.* 2000;14(2):143–5.
 50. Starremans PG, Kersten FF, Knoers NV, van den Heuvel LP, Bindels RJ. Mutations in the human

- Na-K-2Cl cotransporter (NKCC2) identified in Bartter syndrome type I consistently result in nonfunctional transporters. *J Am Soc Nephrol.* 2003;14(6):1419–26.
51. Acuña R, Martínez-de-la-Maza L, Ponce-Coria J, Vázquez N, Ortal-Víte P, Pacheco-Alvarez D, Bobadilla NA, Gamba G. Rare mutations in SLC12A1 and SLC12A3 protect against hypertension by reducing the activity of renal salt cotransporters. *J Hypertens.* 2011;29(3):475–83.
 52. Monette MY, Rinehart J, Lifton RP, Forbush B. Rare mutations in the human Na-K-Cl cotransporter (NKCC2) associated with lower blood pressure exhibit impaired processing and transport function. *Am J Physiol Renal Physiol.* 2011;300(4):F840–7.
 53. Schwalbe RA, Bianchi L, Accili EA, Brown AM. Functional consequences of ROMK mutants linked to antenatal Bartter's syndrome and implications for treatment. *Hum Mol Genet.* 1998;7(6):975–80.
 54. Peters M, Ermert S, Jeck N, Derst C, Pechmann U, Weber S, Schlingmann KP, Seyberth HW, Waldegger S, Konrad M. Classification and rescue of ROMK mutations underlying hyperprostaglandin E syndrome/antenatal Bartter syndrome. *Kidney Int.* 2003;64(3):923–32.
 55. Fang L, Li D, Welling PA. Hypertension resistance polymorphisms in ROMK (Kir1.1) alter channel function by different mechanisms. *Am J Physiol Renal Physiol.* 2010;299(6):F1359–64.
 56. Massa G, Proesmans W, Devlieger H, Vandenberghe K, Van Assche A, Eggermont E. Electrolyte composition of the amniotic fluid in Bartter syndrome. *Eur J Obstet Gynecol Reprod Biol.* 1987;24(4):335–40.
 57. Shalev H, Ohaly M, Meizner I, Carmi R. Prenatal diagnosis of Bartter syndrome. *Prenat Diagn.* 1994;14:996–8.
 58. Yang T, Park JM, Arend L, Huang Y, Topaloglu R, Pasumarthy A, Praetorius H, Spring K, Briggs JP, Schnermann J. Low chloride stimulation of prostaglandin E2 release and cyclooxygenase-2 expression in a mouse macula densa cell line. *J Biol Chem.* 2000;275(48):37922–9.
 59. Kömhoff M, Jeck ND, Seyberth HW, Gröne HJ, Nüsing RM, Breyer MD. Cyclooxygenase-2 expression is associated with the renal macula densa of patients with Bartter-like syndrome. *Kidney Int.* 2000;58(6):2420–44.
 60. Reinalter SC, Jeck N, Brochhausen C, Watzler B, Nüsing RM, Seyberth HW, Kömhoff M. Role of cyclooxygenase-2 in hyperprostaglandin E syndrome/antenatal Bartter syndrome. *Kidney Int.* 2002;62(1):253–60.
 61. Konrad M, Weber S. Recent advances in molecular genetics of hereditary magnesium-losing disorders. *J Am Soc Nephrol.* 2003;14(1):249–60.
 62. Dai LJ, Bapty B, Ritchie G, Quamme GA. PGE2 stimulates Mg²⁺ uptake in mouse distal convoluted tubule cells. *Am J Physiol.* 1998;275:F833–9.
 63. Leonhardt A, Timmermanns G, Roth B, Seyberth HW. Calcium homeostasis and hypercalciuria in hyperprostaglandin E syndrome. *J Pediatr.* 1992;120(4 Pt 1):546–54.
 64. Schurman SJ, Bergstrom WH, Shoemaker LR, Welch TR. Angiotensin II reduces calcium uptake into bone. *Pediatr Nephrol.* 2004;19(1):33–5.
 65. Takahashi N, Chernavvsky DR, Gomez RA, Igarashi P, Gitelman HJ, Smithies O. Uncompensated polyuria in a mouse model of Bartter's syndrome. *Proc Natl Acad Sci U S A.* 2000;97:5434–9.
 66. Lorenz JN, Baird NR, Judd LM, Noonan WT, Andringa A, Doetschman T, Manning PA, Liu LH, Miller ML, Shull GE. Impaired renal NaCl absorption in mice lacking the ROMK potassium channel, a model for type II Bartter's syndrome. *J Biol Chem.* 2002;277:37871–80.
 67. Wagner CA, Loffing-Cueni D, Yan Q, Schulz N, Fakitsas P, Carrel M, Wang T, Verrey F, Geibel JP, Giebisch G, Hebert SC, Loffing J. Mouse model of type II Bartter's syndrome. II. Altered expression of renal sodium- and water-transporting proteins. *Am J Physiol Renal Physiol.* 2008;294(6):F1373–80.
 68. Lu M, Leng Q, Egan ME, Caplan MJ, Boulpaep EL, Giebisch GH, Hebert SC. CFTR is required for PKA-regulated ATP sensitivity of Kir1.1 potassium channels in mouse kidney. *J Clin Invest.* 2006;116(3):797–807.
 69. Rodriguez-Soriano J. Bartter's syndrome comes of age. *Pediatrics.* 1999;103:663–4.
 70. Kleta R, Basoglu C, Kuwertz-Bröking E. New treatment options for Bartter's syndrome. *N Engl J Med.* 2000;343:661–2.
 71. Haas NA, Nossal R, Schneider CH, Lewin MA, Ocker V, Holder M, Uhlemann F. Successful management of an extreme example of neonatal hyperprostaglandin-E syndrome (Bartter's syndrome) with the new cyclooxygenase-2 inhibitor rofecoxib. *Pediatr Crit Care Med.* 2003;4(2):249–51.
 72. Fletcher JT, Graf N, Scarman A, Saleh H, Alexander SI. Nephrotoxicity with cyclooxygenase 2 inhibitor use in children. *Pediatr Nephrol.* 2006;21(12):1893–7.
 73. Wong W, Hulton SA, Taylor CM, Raafat F, Lote CJ, Lindop G. A case of neonatal Bartter's syndrome. *Pediatr Nephrol.* 1996;10(4):414–8.
 74. Puricelli E, Bettinelli A, Borsa N, Sironi F, Mattiello C, Tammaro F, Tedeschi S, Bianchetti MG, Italian Collaborative Group for Bartter Syndrome. Long-term follow-up of patients with Bartter syndrome type I and II. *Nephrol Dial Transplant.* 2010;25(9):2976–81.
 75. Landau D, Shalev H, Ohaly M, Carmi R. Infantile variant of Bartter syndrome and sensorineural deafness: a new autosomal recessive disorder. *Am J Med Genet.* 1995;59:454–9.
 76. Jeck N, Reinalter SC, Henne T, Marg W, Mallmann R, Pasel K, Vollmer M, Klaus G, Leonhardt A, Seyberth HW, Konrad M. Hypokalemic salt-losing tubulopathy

- with chronic renal failure and sensorineural deafness. *Pediatrics*. 2001;108, E5.
77. Brennan TM, Landau D, Shalev H, Lamb F, Schutte BC, Walder RY, Mark AL, Carmi R, Sheffield VC. Linkage of infantile Bartter syndrome with sensorineural deafness to chromosome 1p. *Am J Hum Genet*. 1998;62:355–61.
 78. Estevez R, Boettger T, Stein V, Birkenhager R, Otto E, Hildebrandt F, Jentsch TJ. Barttin is a Cl⁻ channel beta-subunit crucial for renal Cl⁻ reabsorption and inner ear K⁺ secretion. *Nature*. 2001;414:558–61.
 79. Shalev H, Ohali M, Kachko L, Landau D. The neonatal variant of Bartter syndrome and deafness: preservation of renal function. *Pediatrics*. 2003;112(3 Pt 1):628–33.
 80. Zaffanello M, Taranta A, Palma A, Bettinelli A, Marseglia GL, Emma F. Type IV Bartter syndrome: report of two new cases. *Pediatr Nephrol*. 2006;21(6):766–70.
 81. Miyamura N, Matsumoto K, Taguchi T, Tokunaga H, Nishikawa T, Nishida K, Toyonaga T, Sakakida M, Araki E. Atypical Bartter syndrome with sensorineural deafness with G47R mutation of the beta-subunit for ClC-Ka and ClC-Kb chloride channels, barttin. *J Clin Endocrinol Metab*. 2003;88(2):781–6.
 82. García-Nieto V, Flores C, Luis-Yanes MI, Gallego E, Villar J, Claverie-Martín F. Mutation G47R in the BSND gene causes Bartter syndrome with deafness in two Spanish families. *Pediatr Nephrol*. 2006;21(5):643–8.
 83. Kitanaka S, Sato U, Maruyama K, Igarashi T. A compound heterozygous mutation in the BSND gene detected in Bartter syndrome type IV. *Pediatr Nephrol*. 2006;21(2):190–3.
 84. Waldegger S, Jeck N, Barth P, Peters M, Vitzthum H, Wolf K, Kurtz A, Konrad M, Seyberth HW. Barttin increases expression and changes current properties of ClC-K channels. *Pflugers Arch*. 2002;444:411–8.
 85. Riazuddin S, Anwar S, Fischer M, Ahmed ZM, Khan SY, Janssen AG, Zafar AU, Scholl U, Husnain T, Belyantseva IA, Friedman PL, Riazuddin S, Friedman TB, Fahlke C. Molecular basis of DFNB73: mutations of BSND can cause nonsyndromic deafness or Bartter syndrome. *Am J Hum Genet*. 2009;85(2):273–80.
 86. Janssen AG, Scholl U, Domeyer C, Nothmann D, Leinenweber A, Fahlke C. Disease-causing dysfunctions of barttin in Bartter syndrome type IV. *J Am Soc Nephrol*. 2009;20(1):45–53.
 87. Fischer M, Janssen AG, Fahlke C. Barttin activates ClC-K channel function by modulating gating. *J Am Soc Nephrol*. 2010;21(8):1281–9.
 88. Jentsch TJ, Poet M, Fuhrmann JC, Zdebik AA. Physiological functions of CLC Cl⁻ channels gleaned from human genetic disease and mouse models. *Annu Rev Physiol*. 2005;67:779–807.
 89. Schlingmann KP, Konrad M, Jeck N, Waldegger P, Reinalter SC, Holder M, Seyberth HW, Waldegger S. Salt wasting and deafness resulting from mutations in two chloride channels. *N Engl J Med*. 2004;350:1314–9.
 90. Nozu K, Inagaki T, Fu XJ, Nozu Y, Kaito H, Kanda K, Sekine T, Igarashi T, Nakanishi K, Yoshikawa N, Iijima K, Matsuo M. Molecular analysis of digenic inheritance in Bartter syndrome with sensorineural deafness. *J Med Genet*. 2008;45(3):182–6.
 91. Rickheit G, Maier H, Strenke N, Andreescu CE, De Zeeuw CI, Muenscher A, Zdebik AA, Jentsch TJ. Endocochlear potential depends on Cl⁻ channels: mechanism underlying deafness in Bartter syndrome IV. *EMBO J*. 2008;27:2907–17.
 92. Nomura N, Tajima M, Sugawara N, Morimoto T, Kondo Y, Ohno M, Uchida K, Mti K, Bachmann S, Soleimani M, Ohta E, Ohta A, Sahara E, Okado T, Rai T, Jentsch TJ, Sasaki S, Uchida S. Generation and analysis of R8L barttin knockin mouse. *Am J Physiol Renal Physiol*. 2011;301:F297–307.
 93. Nomura N, Kamiya K, Ikeda K, Yui N, Chiga M, Sahara E, Rai T, Sakaki S, Uchida S. Treatment with 17-allylamino-17-demethoxygeldanamycin ameliorated symptoms of Bartter syndrome type IV caused by mutated Bsnd in mice. *Biochem Biophys Res Commun*. 2013;441(3):544–9.
 94. Nozu K, Fu XJ, Nakanishi K, Yoshikawa N, Kaito H, Kanda K, Krol RP, Miyashita R, Kamitsuji H, Kanda S, Hayashi Y, Satomura K, Shimizu N, Iijima K, Matsuo M. Molecular analysis of patients with type III Bartter syndrome: picking up large heterozygous deletions with semiquantitative PCR. *Pediatr Res*. 2007;62(3):364–9.
 95. Brochard K, Boyer O, Blanchard A, Loirat C, Niaudet P, Macher MA, Deschenes G, Bensman A, Decramer S, Cochat P, Morin D, Broux F, Caillez M, Guyot C, Novo R, Jeunemaître X, Vargas-Poussou R. Phenotype-genotype correlation in antenatal and neonatal variants of Bartter syndrome. *Nephrol Dial Transplant*. 2009;24(5):1455–64.
 96. Vargas-Poussou R, Dahan K, Kahila D, Venisse A, Riveira-Munoz E, Debaix H, Grisart B, Bridoux F, Unwin R, Moulin B, Haymann JP, Vantyegehem MC, Rigotherier C, Dussol B, Godin M, Nivet H, Dubourg L, Tack Y, Gimenez-Roqueplo AP, Houiller P, Blanchard A, Devuyst O, Jeunemaître X. Spectrum of mutations in Gitelman Syndrome. *J Am Soc Nephrol*. 2011;22:693–703.
 97. Rodríguez-Soriano J, Vallo A, Pérez de Nanclares G, Bilbao JR, Castaño L. A founder mutation in the CLCNKB gene causes Bartter syndrome type III in Spain. *Pediatr Nephrol*. 2005;20(7):891–6.
 98. Keck M, Andirini O, Lahuna O, Burgos J, Cid LP, Sepulveda FV, L'Hoste S, Blanchard A, Vargas-Poussou R, Lourdel S, Teulon J. Novel *CLCNKB* mutations causing Bartter syndrome affect channel surface expression. *Hum Mut*. 2013;34:1269–78.
 99. Zelikovic I, Szargel R, Hawash A, Labay V, Hatib I, Cohen N, Nakhoul F. A novel mutation in the chloride

- channel gene CLCNKB as a cause of Gitelman and Bartter syndromes. *Kidney Int.* 2003;63:24–32.
100. Andriani O, Keck M, L'Hoste S, Briones R, Mansour-Hendili L, Grand T, Sepúlveda FV, Blanchard A, Lourdel S, Vargas-Poussou R, Teulon J. CLCNKB mutations causing mild Bartter syndrome profoundly alter the pH and Ca²⁺ dependence of ClC-Kb channels. *Pflugers Arch.* 2014;466(9):1713–23.
 101. Matsumura Y, Uchida S, Kondo Y, Miyazaki H, Ko SB, Hayama A, Morimoto T, Liu W, Arisawa M, Sasaki S, Marumo F. Overt nephrogenic diabetes insipidus in mice lacking the ClC-K1 chloride channel. *Nat Genet.* 1991;21(1):95–8.
 102. Barlassina C, Dal Fiume C, Lanzani C, Manunta P, Guffanti G, Ruello A, Bianchi G, Del Vecchio L, Macciardi F, Cusi D. Common genetic variants and haplotypes in renal CLCNKA gene are associated to salt-sensitive hypertension. *Hum Mol Genet.* 2007;16(13):1630–8.
 103. Cappola TP, Matkovich SJ, Wang W, van Booven D, Li M, Wang X, Qu L, Sweitzer NK, Fang JC, Reilly MP, Hakonarson H, Nerbonne JM, Dorn 2nd GW. Loss-of-function DNA sequence variant in the CLCNKA chloride channel implicates the cardio-renal axis in interindividual heart failure risk variation. *Proc Natl Acad Sci U S A.* 2011;108(6):2456–61.
 104. Westland R, Hack WW, van der Horst HJ, Uittenbogaard LB, van Hagen JM, van der Valk P, Kamsteeg EJ, van den Heuvel LP, van Wijk JA. Bartter syndrome type III and congenital anomalies of the kidney and urinary tract: an antenatal presentation. *Clin Nephrol.* 2012;78(6):492–6.
 105. Bettinelli A, Borsa N, Bellantuono R, Syrén ML, Calabrese R, Edefonti A, Komminos J, Santostefano M, Beccaria L, Pela I, Bianchetti MG, Tedeschi S. Patients with biallelic mutations in the chloride channel gene CLCNKB: long-term management and outcome. *Am J Kidney Dis.* 2007;49(1):91–8.
 106. Schurman SJ, Perlman SA, Sutphen R, Campos A, Garin EH, Cruz DN, Shoemaker LR. Genotype/phenotype observations in African Americans with Bartter syndrome. *J Pediatr.* 2001;139(1):105–10.
 107. Adachi M, Tajima T, Muroya K, Asakura Y. Classic Bartter syndrome complicated with profound growth hormone deficiency: a case report. *J Med Case Rep.* 2013;7:283.
 108. Jeck N, Konrad M, Peters M, Weber S, Bonzel KE, Seyberth HW. Mutations in the chloride channel gene, CLCNKB, leading to a mixed Bartter-Gitelman phenotype. *Pediatr Res.* 2000;48:754–8.
 109. Fukuyama S, Hiramatsu M, Akagi M, Higa M, Ohta T. Novel mutations of the chloride channel Kb gene in two Japanese patients clinically diagnosed as Bartter syndrome with hypocalciuria. *J Clin Endocrinol Metab.* 2004;89(11):5847–50.
 110. Sun H, Demirci H, Shields CL, Shields JA. Sclerochoroidal calcification in a patient with classic Bartter's syndrome. *Am J Ophthalmol.* 2005;139:365–6.
 111. Robitaille P, Merouani A, He N, Pei Y. Bartter syndrome in two sisters with a novel mutation of the CLCNKB gene, one with deafness. *Eur J Pediatr.* 2011;170(9):1209–11.
 112. Walker SH. Severe Bartter syndrome in blacks. *N Engl J Med.* 1971;285(20):1150.
 113. Calò LA. Vascular tone control in humans: insights from studies in Bartter's/Gitelman's syndromes. *Kidney Int.* 2006;69(6):963–6.
 114. Stoff JS, Stemerman M, Steer M, Salzman E, Brown RS. A defect in platelet aggregation in Bartter's syndrome. *Am J Med.* 1980;68(2):171–80.
 115. Clive DM, Stoff JS, Cardi M, MacIntyre DE, Brown RS, Salzman EW. Evidence that circulating 6keto prostaglandin E1 causes the platelet defect of Bartter's syndrome. *Prostaglandins Leukot Essent Fatty Acids.* 1990;41(4):251–8.
 116. Sardani Y, Qin K, Haas M, Aronson AJ, Rosenfield RL. Bartter syndrome complicated by immune complex nephropathy. Case report and literature review. *Pediatr Nephrol.* 2003;18:913–8.
 117. Blethen SL, Van Wyck JJ, Lorentz WB, Jennette JC. Reversal of Bartter's syndrome by renal transplantation in a child with focal, segmental glomerular sclerosis. *Am J Med Sci.* 1985;289:31–6.
 118. Su IH, Frank R, Gauthier BG, Valderrama E, Simon DB, Lifton RP, Trachtman H. Bartter syndrome and focal segmental glomerulosclerosis: a possible link between two diseases. *Pediatr Nephrol.* 2000;14:970–2.
 119. Bartter FC. So-called Bartter's syndrome. *N Engl J Med.* 1969;281(26):1483–4.
 120. Takahashi M, Yanagida N, Okano M, Ishizaki A, Meguro J, Kukita K, Tamaki T, Yonekawa M, Kawamura A, Yokoyama T. A first report: living related kidney transplantation on a patient with Bartter's syndrome. *Transplant Proc.* 1996;28(3):1588.
 121. Watanabe T, Tajima T. Renal cysts and nephrocalcinosis in a patient with Bartter syndrome type III. *Pediatr Nephrol.* 2005;20(5):676–8.
 122. Torres VE, Young Jr WF, Offord KP, Hattery RR. Association of hypokalemia, aldosteronism, and renal cysts. *N Engl J Med.* 1990;322(6):345–51.
 123. Jeck N, Waldegger P, Doroszewicz J, Seyberth H, Waldegger S. A common sequence variation of the CLCNKB gene strongly activates ClC-Kb chloride channel activity. *Kidney Int.* 2004;65:190–7.
 124. Jeck N, Waldegger S, Lampert A, Boehmer C, Waldegger P, Lang PA, Wissinger B, Friedrich B, Risler T, Moehle R, Lang UE, Zill P, Bondy B, Schaeffeler E, Asante-Poku S, Seyberth H, Schwab M, Lang F. Activating mutation of the renal epithelial chloride channel ClC-Kb predisposing to hypertension. *Hypertension.* 2004;43(6):1175–81.
 125. Geller DS. A genetic predisposition to hypertension? *Hypertension.* 2004;44:27–8.

126. Kokubo Y, Iwai N, Tago N, Inamoto N, Okayama A, Yamawaki H, Naraba H, Tomoike H. Association analysis between hypertension and CYBA, CLCNKB, and KCNNB1 functional polymorphisms in the Japanese population-the Suita study. *Circ J*. 2005;69(2):138–42.
127. Meyers AM, Feldman C, Sonnekus MI, Ninin DT, Margolius LP, Whalley NA. Chronic laxative abusers with pseudo-idiopathic oedema and autonomous pseudo-Bartter's syndrome. A spectrum of metabolic madness, or new lights on an old disease? *S Afr Med J*. 1990;78(11):631–6.
128. D'Avanzo M, Santinelli R, Tolone C, Bettinelli A, Bianchetti MG. Concealed administration of frusemide simulating Bartter syndrome in a 4.5-year-old boy. *Pediatr Nephrol*. 1995;9(6):749–50.
129. Ramos E, Hall-Craggs M, Demers LM. Surreptitious habitual vomiting simulating Bartter's syndrome. *JAMA*. 1980;243(10):1070–2.
130. Colussi G, Rombolà G, Airaghi C, De Ferrari ME, Minetti L. Pseudo-Bartter's syndrome from surreptitious diuretic intake: differential diagnosis with true Bartter's syndrome. *Nephrol Dial Transplant*. 1992;7(9):896–901.
131. Gladziwa U, Schwarz R, Gitter AH, Bijman J, Seyberth H, Beck F, Ritz E, Gross P. Chronic hypokalaemia of adults: Gitelman's syndrome is frequent but classical Bartter's syndrome is rare. *Nephrol Dial Transplant*. 1995;10(9):1607–13.
132. Whyte MP, Shaheb S, Schnaper HW. Cystinosis presenting with features suggesting Bartter syndrome. Case report and literature review. *Clin Pediatr (Phila)*. 1985;24(8):447–51.
133. Emma F, Pizzini C, Tessa A, Di Giandomenico S, Onetti-Muda A, Santorelli FM, Bertini E, Rizzoni G. "Bartter-like" phenotype in Kearns-Sayre syndrome. *Pediatr Nephrol*. 2006;21(3):355–60.
134. Walker SH, Firminger HI. Familial renal dysplasia with sodium wasting and hypokalemic alkalosis. *Am J Dis Child*. 1974;127(6):882–7.
135. Kennedy JD, Dinwiddie R, Daman-Willems C, Dillon MJ, Matthew DJ. Pseudo-Bartter's syndrome in cystic fibrosis. *Arch Dis Child*. 1990;65(7):786–7.
136. Bates CM, Baum M, Quigley R. Cystic fibrosis presenting with hypokalemia and metabolic alkalosis in a previously healthy adolescent. *J Am Soc Nephrol*. 1997;8(2):352–5.
137. Koshida R, Sakazume S, Maruyama H, Okuda N, Ohama K, Asano S. A case of pseudo-Bartter's syndrome due to intestinal malrotation. *Acta Paediatr Jpn*. 1994;36(1):107–11.
138. Vanhaesebrouck S, Van Laere D, Frysens JP, Theyskens C. Pseudo-Bartter syndrome due to Hirschsprung disease in a neonate with an extra ring chromosome 8. *Am J Med Genet A*. 2007;143A(20):2469–72.
139. Langhendries JP, Thiry V, Bodart E, Delfosse G, Whitofs L, Battisti O, Bertrand JM. Exogenous prostaglandin administration and pseudo-Bartter syndrome. *Eur J Pediatr*. 1989;149(3):208–9.
140. Landau D, Kher KK. Gentamicin-induced Bartter-like syndrome. *Pediatr Nephrol*. 1997;11(6):737–40.
141. Chou CL, Chen YH, Chau T, Lin SH. Acquired Bartter-like syndrome associated with gentamicin administration. *Am J Med Sci*. 2005;329:144–9.
142. Lieber IH, Stoneburner SD, Floyd M, McGuffin WL. Potassium-wasting nephropathy secondary to chemotherapy simulating Bartter's syndrome. *Cancer*. 1984;54(5):808–10.
143. Pedro-Botet J, Tomas S, Soriano JC, Coll J. Primary Sjögren's syndrome associated with Bartter's syndrome. *Clin Exp Rheumatol*. 1991;9(2):210–2.
144. Casatta L, Ferraccioli GF, Bartoli E. Hypokalaemic alkalosis, acquired Gitelman's and Bartter's syndrome in chronic sialoadenitis. *Br J Rheumatol*. 1997;36(10):1125–8.
145. Güllner HG, Bartter FC, Gill Jr JR, Dickman PS, Wilson CB, Tiwari JL. A sibship with hypokalemic alkalosis and renal proximal tubulopathy. *Arch Intern Med*. 1983;143(8):1534–40.
146. Ertekin V, Selimoglu AM, Orbak Z. Association of Bartter's syndrome and empty sella. *J Pediatr Endocrinol Metab*. 2003;16(7):1065–8.
147. Addolorato G, Ancarani F, Leggio L, Abenavoli L, de Lorenzi G, Montalto M, Staffolani E, Zannoni GF, Costanzi S, Gasbarrini G. Hypokalemic nephropathy in an adult patient with partial empty sella: a classic Bartter's syndrome, a Gitelman's syndrome or both? *Panminerva Med*. 2006;48(2):137–42.
148. Jest P, Pedersen KE, Klitgaard NA, Thomsen N, Kjaer K, Simonsen E. Angiotensin-converting enzyme inhibition as a therapeutic principle in Bartter's syndrome. *Eur J Clin Pharmacol*. 1991;41(4):303–5.
149. Kim JY, Kim GA, Song JH, Lee SW, Han JY, Lee JS, Kim MJ. A case of living-related kidney transplantation in Bartter's syndrome. *Yonsei Med J*. 2000;41(5):662–5.
150. Brimacombe JR, Breen DP. Anesthesia and Bartter's syndrome: a case report and review. *AANA J*. 1993;61(2):193–7.
151. Vetrugno L, Cheli G, Bassi F, Giordano F. Cardiac anesthesia management of a patient with Bartter's syndrome. *J Cardiothorac Vasc Anesth*. 2005;19(3):373–6.
152. Gitelman HJ, Graham JB, Welt LG. A new familial disorder characterized by hypokalemia and hypomagnesemia. *Trans Assoc Am Physicians*. 1966;79:221–35.
153. Bettinelli A, Bianchetti MG, Girardin E, Caringella A, Cecconi M, Appiani AC, Pavanello L, Gastaldi R, Isimbaldi C, Lama G, et al. Use of calcium excretion values to distinguish two forms of primary renal tubular hypokalemic alkalosis: Bartter and Gitelman syndromes. *J Pediatr*. 1992;120:38–43.
154. Sutton RA, Mavichak V, Halabe A, Wilkins GE. Bartter's syndrome: evidence suggesting a distal tubular defect in a hypocalciuric variant of the syndrome. *Miner Electrolyte Metab*. 1992;18(1):43–51.

155. Tsukamoto T, Kobayashi T, Kawamoto K, Fukase M, Chihara K. Possible discrimination of Gitelman's syndrome from Bartter's syndrome by renal clearance study: report of two cases. *Am J Kidney Dis.* 1995; 25(4):637–41.
156. Hebert SC, Mount DB GG. Molecular physiology of cation-coupled Cl⁻ cotransport: the SLC12 family. *Pfluegers Arch.* 2004;447:580–93.
157. Gamba G, Saltzberg SN, Lombardi M, Miyanoshiba A, Lytton J, Ma H, Brenner BM, Hebert SC. Primary structure and functional expression of a cDNA encoding the thiazide-sensitive, electroneutral sodium-chloride cotransporter. *Proc Natl Acad Sci U S A.* 1993;90:2749–53.
158. Moreno E, Cristóbal PS, Rivera M, Vázquez N, Bobadilla NA, Gamba G. Affinity-defining domains in the Na-Cl cotransporter: a different location for Cl⁻ and thiazide binding. *J Biol Chem.* 2006;281(25): 17266–75.
159. Takeuchi Y, Mishima E, Shima H, Akiyama Y, Suzuki C, Suzuki T, Kobayashi T, Suzuki Y, Nakayama T, Takeshima Y, Vazquez N IS, Gamba G, Abe T. Exonic mutations in the SLC12A3 gene cause exon skipping and premature termination in Gitelman Syndrome. *J Am Soc Nephrol.* 2014;26:1–9.
160. Lemmink HH, Knoers NV, Karolyi L, van Dijk H, Niaudet P, Antignac C, Guay-Woodford LM, Goodyer PR, Carel JC, Hermes A, Seyberth HW, Monnens LA, van den Heuvel LP. Novel mutations in the thiazide-sensitive NaCl cotransporter gene in patients with Gitelman syndrome with predominant localization to the C-terminal domain. *Kidney Int.* 1998;54:720–30.
161. Reissinger A, Ludwig M, Utsch B, Prömse A, Baulmann J, Weisser B, Vetter H, Kramer HJ, Bokemeyer D. Novel NCCT gene mutations as a cause of Gitelman's syndrome and a systematic review of mutant and polymorphic NCCT alleles. *Kidney Blood Press Res.* 2002;25:354–62.
162. Riveira-Munoz E, Chang Q, Godefroid N, Hoenderop JG, Bindels RJ, Dahan K, Devuyst O, Belgian Network for Study of Gitelman Syndrome. Transcriptional and functional analyses of SLC12A3 mutations: new clues for the pathogenesis of Gitelman syndrome. *J Am Soc Nephrol.* 2007; 18(4):1271–83.
163. Nozu K, Iijima K, Nozu Y, Ikegami E, Imai T, Jun Fu X, Kaito H, Nakanishi K, Yoshikawa N, Matsuo M. A deep intronic mutation in the *SLC12A3* gene leads to Gitelman syndrome. *Pediatr Res.* 2009; 66:590–3.
164. Lo YF, Nozu K, Iijima K, Morishita T, Huang CC, Yang SS, Sytwu HK, Fang YW, Tseng MH, Lin SH. Recurrent deep intronic mutations in the *SLC12A3* gene responsible for Gitelman Syndrome. *Clin J Am Soc Nephrol.* 2011;6:630–9.
165. Riveira-Munoz E, Devuyst O, Belge H, Jeck N, Strompf L, Vargas-Poussou R, Jeunemaitre X, Blanchard A, Knoers NV, Konrad M, Dahan K. Evaluating PVALB as a candidate gene for SLC12A3-negative cases of Gitelman's syndrome. *Nephrol Dial Transplant.* 2008;23:3120–5.
166. Kunchaparty S, Palco M, Berkman J, Velazquez H, Desir GV, Bernstein P, Reilly RF, Ellison DH. Defective processing and expression of thiazide-sensitive Na-Cl cotransporter as a cause of Gitelman's syndrome. *Am J Physiol.* 1999;277:F643–9.
167. de Jong JC, van der Vliet WA, van den Heuvel L, Willems PHGM, Knoers NVAM, Bindels RJM. Functional expression of mutations in the human NaCl cotransporter: evidence for impaired routing mechanisms in Gitelman's syndrome. *J Am Soc Nephrol.* 2002;13:1442–8.
168. Sabath E, Meade P, Berkman J, De los Heros P, Moreno E, Bobadilla NA, Vazquez N, Ellison DH, Gamba G. Pathophysiology of functional mutations of the thiazide-sensitive Na-Cl cotransporter in Gitelman disease. *Am J Physiol Renal Physiol.* 2004;287:F195–203.
169. Schultheis PJ, Lorenz JN, Meneton P, Nieman ML, Riddle TM, Flagella M, Duffy JJ, Doetschman T, Miller ML, Shull GE. Phenotype resembling Gitelman's syndrome in mice lacking the apical Na⁺-Cl⁻ cotransporter of the distal convoluted tubule. *J Biol Chem.* 1998;273:29150–5.
170. Loffing J, Vallon V, Loffing-Cueni D, Aregger F, Richter K, Pietri L, Bloch-Faure M, Hoenderop JG, Shull GE, Meneton P, Kaissling B. Altered renal distal tubule structure and renal Na⁺ and Ca²⁺ handling in a mouse model for Gitelman's syndrome. *J Am Soc Nephrol.* 2004;15:2276–88.
171. Morris RG, Hoorn EJ, Knepper MA. Hypokalemia in a mouse model of Gitelman's syndrome. *Am J Physiol Renal Physiol.* 2006;290:F1416–20.
172. Belge H, Gailly P, Schwaller B, Loffing J, Debaix H, Riveira-Munoz E, Beauwens R, Devogelaer JP, Hoenderop JG, Bindels RJ, Devuyst O. Renal expression of parvalbumin is critical for NaCl handling and response to diuretics. *Proc Natl Acad Sci U S A.* 2007;104(37):14849–54.
173. Nijenhuis T, Hoenderop JG, Loffing J, van der Kemp AW, van Os CH, Bindels RJ. Thiazide-induced hypocalcemia is accompanied by a decreased expression of Ca²⁺ transport proteins in kidney. *Kidney Int.* 2003;64(2):555–64.
174. Hoenderop JG, van der Kemp AW, Hartog A, van Os CH, Willems PH, Bindels RJ. The epithelial calcium channel, ECaC, is activated by hyperpolarization and regulated by cytosolic calcium. *Biochem Biophys Res Commun.* 1999;261(2):488–92.
175. Ellison DH. Divalent cation transport by the distal nephron: insights from Bartter's and Gitelman's syndromes. *Am J Physiol Renal Physiol.* 2000;279(4):F616–25.
176. Nijenhuis T, Vallon V, van der Kemp AW, Loffing J, Hoenderop JG, Bindels RJ. Enhanced passive Ca²⁺ reabsorption and reduced Mg²⁺ channel abundance explains thiazide-induced hypocalcemia and hypomagnesemia. *J Clin Invest.* 2005;115:1651–8.

177. Reilly RF, Huang CL. The mechanism of hypocalciuria with NaCl cotransporter inhibition. *Nat Rev Nephrol.* 2011;7:669–74.
178. Dai LJ, Ritchie G, Kerstan D, Kang HS, Cole DE, Quamme GA. Magnesium transport in the renal distal convoluted tubule. *Physiol Rev.* 2001;81:51–84.
179. Schlingmann KP, Weber S, Peters M, Nejsum LN, Vitzthum H, Klingel K, Kratz M, Haddad E, Ristoff E, Dinour D, Syrrou M, Nielsen S, Sassen M, Waldegger S, Seyberth HW, Konrad M. Hypomagnesemia with secondary hypocalcemia is caused by mutations in TRPM6, a new member of the TRPM gene family. *Nat Genet.* 2002;31:166–70.
180. Loffing J, Loffing-Cueni D, Hegyi I, Kaplan MR, Hebert SC, Le Hir M, Kaissling B. Thiazide treatment of rats provokes apoptosis in distal tubule cells. *Kidney Int.* 1996;50:1180–90.
181. Yang SS, Lo YF, Wu CC, Lin SW, Yeh CJ, Chu P, Sytwu HK, Uchida S, Sasaki S, Lin SH. SPAK-knockout mice manifest Gitelman syndrome and impaired vasoconstriction. *J Am Soc Nephrol.* 2010;21(11):1868–77.
182. Godefroid N, Riveira-Munoz E, Saint-Martin C, Nassogne MC, Dahan K, Devuyt O. A novel splicing mutation in SLC12A3 associated with Gitelman syndrome and idiopathic intracranial hypertension. *Am J Kidney Dis.* 2006;48(5):e73–9.
183. Knoers NV. Gitelman syndrome. *Adv Chronic Kidney Dis.* 2006;13(2):148–54.
184. Cruz DN, Simon DB, Nelson-Williams C, Farhi A, Finberg K, Bursleson L, Gill JR, Lifton RP. Mutations in the Na-Cl cotransporter reduce blood pressure in humans. *Hypertension.* 2001;37(6):1458–64.
185. Balavoine AS, Bataille P, Vanhille P, Azar R, Noël C, Asseman P, Soudan B, Wémeau JL, Vantyghem MC. Phenotype-genotype correlation and follow-up in adult patients with hypokalemia of renal origin suggesting Gitelman syndrome. *Eur J Endocrinol.* 2011;165:665–73.
186. Berry MR, Robinson C, Karte Frankl FE. Unexpected clinical sequelae of Gitelman syndrome: hypertension in adulthood is common and females have higher potassium requirements. *Nephrol Dial Transplant.* 2013;28:1533–42.
187. Punzi L, Calò L, Schiavon F, Pionon M, Rosada M, Todesco S. Chondrocalcinosis is a feature of Gitelman's variant of Bartter's syndrome. A new look at the hypomagnesemia associated with calcium pyrophosphate dihydrate crystal deposition disease. *Rev Rhum Engl Ed.* 1998;65(10):571–4.
188. Bourcier T, Blain P, Massin P, Grünfeld JP, Gaudric A. Sclerochoroidal calcification associated with Gitelman syndrome. *Am J Ophthalmol.* 1999;128(6):767–78.
189. Nicolet-Barousse L, Blanchard A, Roux C, Pietri L, Bloch-Faure M, Kolta S, Chappard C, Geoffroy V, Morieux C, Jeunemaitre X, Shull GE, Meneton P, Paillard M, Houillier P, De Vernejoul MC. Inactivation of the Na-Cl co-transporter (NCC) gene is associated with high BMD through both renal and bone mechanisms: analysis of patients with Gitelman syndrome and Ncc null mice. *J Bone Miner Res.* 2005;20(5):799–808.
190. Bettinelli A, Tosetto C, Colussi G, Tommasini G, Edefonti A, Bianchetti MG. Electrocardiogram with prolonged QT interval in Gitelman disease. *Kidney Int.* 2002;62(2):580–4.
191. Foglia PE, Bettinelli A, Tosetto C, Cortesi C, Crosazzo L, Edefonti A, Bianchetti MG. Cardiac work up in primary renal hypokalaemia-hypomagnesaemia (Gitelman syndrome). *Nephrol Dial Transplant.* 2004;19(6):1398–402.
192. Pachulski RT, Lopez F, Sharaf R. Gitelman's not so benign syndrome. *N Engl J Med.* 2005;353:850–1.
193. Srinivas SK, Sukhan S, Elovitz MA. Nausea, emesis, and muscle weakness in a pregnant adolescent. *Obstet Gynecol.* 2006;107(2 Pt 2):481–4.
194. von Vigier RO, Ortisi MT, la Manna A, Bianchetti MG, Bettinelli A. Hypokalemic rhabdomyolysis in congenital tubular disorders: a case series and a systematic review. *Pediatr Nephrol.* 2010;25:861–6.
195. Kumagai H, Matsumoto S, Nozu K. Hypokalemic rhabdomyolysis in a child with Gitelman's syndrome. *Pediatr Nephrol.* 2010;25:953–5.
196. Cortesi C, Lava SA, Bettinelli A, Tammaro F, Giannini O, Caiata-Zufferey M, Bianchetti MG. Cardiac arrhythmias and rhabdomyolysis in Bartter-Gitelman patients. *Pediatr Nephrol.* 2010;25(10):2005–8.
197. Ducarme G, Davitian C, Uzan M, Belenfant X, Poncet C. Pregnancy in a patient with Gitelman syndrome: a case report and review of literature. *J Gynecol Obstet Biol Reprod (Paris).* 2007;36(3):310–3.
198. Bianchetti MG, Edefonti A, Bettinelli A. The biochemical diagnosis of Gitelman disease and the definition of "hypocalciuria". *Pediatr Nephrol.* 2003;18(5):409–11.
199. Colussi G, Bettinelli A, Tedeschi S, De Ferrari ME, Syrén ML, Borsa N, Mattiello C, Casari G, Bianchetti MG. A thiazide test for the diagnosis of renal tubular hypokalemic disorders. *Clin J Am Soc Nephrol.* 2007;2(3):454–60.
200. Viganò C, Amoroso C, Barretta F, Minnici G, Albisetti W, Syrén ML, Bianchetti MG, Bettinelli A. Renal phosphate handling in Gitelman syndrome – the results of a case-control study. *Pediatr Nephrol.* 2013;28:65–70.
201. Azak A, Huddam B, Koçak G, Ortobozkoyum L, Uzel M, Duranay M. Gitelman syndrome complicated with dysglycemia. *Acta Diabetol.* 2011;48:249–50.
202. Tseng MH, Yang SS, Hsu YJ, Fang YW, Wu CJ, Tsai JD, Hwang DY, Lin SH. Genotype, phenotype, and follow-up in Taiwanese patients with salt-losing tubulopathy associated with *SLC12A3* mutation. *J Clin Endocrinol Metab.* 2012;97(8):E1478–82.
203. Ren H, Qin L, Wang W MJ, Zhang W, Shen PY, Shi H, Li X, Chen N. Abnormal glucose metabolism and

- insulin sensitivity in Chinese patients with Gitelman syndrome. *Am J Nephrol.* 2013;37(2):152–7.
204. Joo KW, Lee JW, Jang HR, Heo NJ, Jeon US, Oh YK, Lim CS, Na KY, Kim J, Cheong HI, Han JS. Reduced urinary excretion of thiazide-sensitive Na-Cl cotransporter in Gitelman syndrome: preliminary data. *Am J Kidney Dis.* 2007;50(5):765–73.
 205. Bulucu F, Vural A, Yenicesu M, Caglar K. Association of Gitelman's syndrome and focal segmental glomerulosclerosis. *Nephron.* 1998;79:244.
 206. Hanevold C, Mian A, Dalton R. C1q nephropathy in association with Gitelman syndrome: a case report. *Pediatr Nephrol.* 2006;21(12):1904–8.
 207. Ceri M, Unverdi S, Altay M, Unverdi H, Kurultak I, Yilmaz R, Ensari A, Duranay M. Focal segmental glomerulosclerosis in association with Gitelman syndrome. *Int Urol Nephrol.* 2011;43:905–7.
 208. Demoulin N, Aydin S, Cosyns JP, Dahan K, Cornet G, Auberger I, Loffing J, Devuyst O. Gitelman syndrome and glomerular proteinuria: a link between loss of sodium-chloride cotransporter and podocyte dysfunction? *Nephrol Dial Transplant.* 2014;29:iv117–20.
 209. Cruz DN, Shaer AJ, Bia MJ, Lifton RP, Simon DB; Yale Gitelman's and Bartter's Syndrome Collaborative Study Group. Gitelman's syndrome revisited: an evaluation of symptoms and health-related quality of life. *Kidney Int.* 2001;59:710–7.
 210. Coto E, Rodriguez J, Jeck N, Alvarez V, Stone R, Loris C, Rodriguez LM, Fischbach M, Seybert HW, Santos F. A new mutation (intron 9 +1 G>T) in the SLC12A3 gene is linked to Gitelman syndrome in Gypsies. *Kidney Int.* 2004;65:25–9.
 211. Lin SH, Cheng NL, Hsu YJ, Halperin ML. Intrafamilial phenotype variability in patients with Gitelman syndrome having the same mutations in their thiazide-sensitive sodium/chloride cotransporter. *Am J Kidney Dis.* 2004;43:304–12.
 212. Riveira-Munoz E, Chang Q, Bindels RJ, Devuyst O. Gitelman syndrome: towards genotype-phenotype correlations? *Pediatr Nephrol.* 2007;22:326–32.
 213. Hu DC, Burtner C, Hong A, Lobo PI, Okusa MD. Correction of renal hypertension after kidney transplantation from a donor with Gitelman syndrome. *Am J Med Sci.* 2006;331(2):105–9.
 214. Sassen MC, Jeck N, Klaus G. Can renal tubular hypokalemic disorders be accurately diagnosed on the basis of the diuretic response to thiazide? *Nat Clin Pract Nephrol.* 2007;3(10):528–9.
 215. Panichpisal K, Angulo-Pernett F, Selhi S, Nugent KM. Gitelman-like syndrome after cisplatin therapy: a case report and literature review. *BMC Nephrol.* 2006;7:10.
 216. Arany I, Safirstein RL. Cisplatin nephrotoxicity. *Semin Nephrol.* 2003;23(5):460–4.
 217. Ren H, Wang WM, Chen XN, Zhang W, Pan XX, Wang XL, Lin Y, Zhang S, Chen N. Renal involvement and followup of 130 patients with primary Sjögren's syndrome. *J Rheumatol.* 2008;35(2):278–84.
 218. Persu A, Lafontaine JJ, Devuyst O. Chronic hypokalaemia in young women – it is not always abuse of diuretics. *Nephrol Dial Transplant.* 1999;14(4):1021–5.
 219. Schwarz C, Barisani T, Bauer E, Druml W. A woman with red eyes and hypokalemia: a case of acquired Gitelman syndrome. *Wien Klin Wochenschr.* 2006;118(7–8):239–42.
 220. Rodríguez-Soriano J. Bartter and related syndromes: the puzzle is almost solved. *Pediatr Nephrol.* 1998;12(4):315–27.
 221. Shaer AJ. Inherited primary renal tubular hypokalemic alkalosis: a review of Gitelman and Bartter syndromes. *Am J Med Sci.* 2001;322(6):316–32.
 222. Colussi G, Rombolà G, De Ferrari ME, Macaluso M, Minetti L. Correction of hypokalemia with antialdosterone therapy in Gitelman's syndrome. *Am J Nephrol.* 1994;14(2):127–35.
 223. Morton A. Eplerenone in the treatment of Gitelman syndrome. *Intern Med J.* 2008;38:377.
 224. Morton A, Panitz B, Bush A. Eplerenone for Gitelman syndrome in pregnancy. *Nephrology.* 2011;16:349–50.
 225. Ito Y, Yoshida M, Nakayama M, Tsutaya S, Ogawa K, Maeda H, Miyata M, Oiso Y. Eplerenone improved hypokalemia in a patient with Gitelman's syndrome. *Intern Med.* 2012;51(1):83–6.
 226. Blanchard A, Vargas-Poussou R, Valle M, Caumont-Prim A, Allard J, Desport E, Dubourg L, Monge M, Bergerot D, Baron S, Essig M, Bridoux F, Tack I, Azizi M. Indomethacin, amiloride, or eplerenone for treating hypokalemia in Gitelman syndrome. *J Am Soc Nephrol.* 2014;26:1–8.
 227. Zannad F, McMurray JJ, Krum H, Krum H, van Veldhuisen DJ, Swedberg K, Shi H, Vincent J, Pocock SJ, Pitt B. Eplerenone in patients with systolic heart failure and mild symptoms. *N Engl J Med.* 2011;364:11–21.
 228. Liaw LC, Banerjee K, Coulthard MG. Dose related growth response to indomethacin in Gitelman syndrome. *Arch Dis Child.* 1999;81(6):508–10.
 229. Mayan H, Gurevitz O, Farfel Z. Successful treatment by cyclooxygenase-2 inhibitor of refractory hypokalemia in a patient with Gitelman's syndrome. *Clin Nephrol.* 2002;58(1):73–6.
 230. Bettinelli A, Metta MG, Perini A, Basilico E, Santeramo C. Long-term follow-up of a patient with Gitelman's syndrome. *Pediatr Nephrol.* 1993;7(1):67–8.
 231. Bonfante L, Davis PA, Spinello M, Antonello A, D'Angelo A, Semplicini A, Calò L. Chronic renal failure, end-stage renal disease, and peritoneal dialysis in Gitelman's syndrome. *Am J Kidney Dis.* 2001;38(1):165–8.
 232. Calò LA, Marchini F, Davis PA, Rigotti P, Pagnin E, Semplicini A. Kidney transplant in Gitelman's syndrome. Report of the first case. *J Nephrol.* 2003;16(1):144–7.

233. Brown EM, Gamba G, Riccardi D, Lombardi M, Butters R, Kifor O, Sun A, Hediger MA, Lytton J, Hebert SC. Cloning and characterization of an extracellular Ca^{2+} -sensing receptor from bovine parathyroid. *Nature*. 1993;366(6455):575–80.
234. Garrett JE, Capuano IV, Hammerland LG, Hung BC, Brown EM, Hebert SC, Nemeth EF, Fuller F. Molecular cloning and functional expression of human parathyroid calcium receptor cDNAs. *J Biol Chem*. 1995;270(21):12919–25.
235. Bai M, Trivedi S, Kifor O, Quinn SJ, Brown EM. Intermolecular interactions between dimeric calcium-sensing receptor monomers are important for its normal function. *Proc Natl Acad Sci USA*. 1999;96:2834–9.
236. Brown EM, MacLeod RJ. Extracellular calcium sensing and extracellular calcium signaling. *Physiol Rev*. 2001;81:239–97.
237. Bapty BW, Dai LJ, Ritchie G, Canaff L, Hendy GN, Quamme GA. $\text{Mg}^{2+}/\text{Ca}^{2+}$ sensing inhibits hormone-stimulated Mg^{2+} uptake in mouse distal convoluted tubule cells. *Am J Physiol*. 1998;275:F353–60.
238. Quamme GA. Control of magnesium transport in the thick ascending limb. *Am J Physiol*. 1989;256(2 Pt 2):197–210.
239. Hebert SC, Brown EM, Harris HW. Role of the Ca^{2+} -sensing receptor in divalent mineral ion homeostasis. *J Exp Biol*. 1997;200(Pt 2):295–302.
240. Thakker RV. Diseases associated with the extracellular calcium-sensing receptor. *Cell Calcium*. 2004;35(3):275–82.
241. Chou YH, Brown EM, Levi T, Crowe G, Atkinson AB, Armqvist HJ, Toss G, Fuleihan GE, Seidman JG, Seidman CE. The gene responsible for familial hypocalciuric hypercalcemia maps to chromosome 3q in four unrelated families. *Nat Genet*. 1992;1(4):295–300.
242. Pollak MR, Brown EM, Chou YH, Hebert SC, Steinmann B, Levi T, Seidman CE, Seidman JG. Mutations in the human Ca^{2+} -sensing receptor gene cause familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. *Cell*. 1993;75:1297–303.
243. Pollak MR, Brown EM, Estep HL, McLaine PN, Kifor O, Park J, Hebert SC, Seidman CE, Seidman JG. Autosomal dominant hypocalcaemia caused by a Ca^{2+} -sensing receptor gene mutation. *Nat Genet*. 1994;8:303–7.
244. Pearce SH, Williamson C, Kifor O, Bai M, Coulthard MG, Davies M, Lewis-Barned N, McCredie D, Powell H, Kendall-Taylor P, Brown EM, Thakker RV. A familial syndrome of hypocalcemia with hypercalciuria due to mutations in the calcium-sensing receptor. *N Engl J Med*. 1996;335:1115–22.
245. Watanabe S, Fukumoto S, Chang H, Takeuchi Y, Hasegawa Y, Okazaki R, Chikatsu N, Fujita T. Association between activating mutations of calcium-sensing receptor and Bartter's syndrome. *Lancet*. 2002;360:692–4.
246. Vargas-Poussou R, Huang C, Hulin P, Houillier P, Jeunemaitre X, Paillard M, Planelles G, Dechaux M, Miller RT, Antignac C. Functional characterization of a calcium-sensing receptor mutation in severe autosomal dominant hypocalcemia with a Bartterlike syndrome. *J Am Soc Nephrol*. 2002;13:2259–66.
247. Gunn IR, Gaffney D. Clinical and laboratory features of calcium-sensing receptor disorders: a systematic review. *Ann Clin Biochem*. 2004;41(Pt 6):441–58.
248. Foley Jr TP, Harrison HC, Arnaud CD, Harrison HE. Familial benign hypercalcemia. *J Pediatr*. 1972;6:1060–7.
249. Marx SJ, Attie MF, Levine MA, Spiegel AM, Downs Jr RW, Lasker RD. The hypocalciuric or benign variant of familial hypercalcemia: clinical and biochemical features in fifteen kindreds. *Medicine (Baltimore)*. 1981;60:397–412.
250. Heath 3rd H. Familial benign (hypocalciuric) hypercalcemia. A troublesome mimic of mild primary hyperparathyroidism. *Endocrinol Metab Clin North Am*. 1989;3:723–40.
251. Bilezikian JP, Potts Jr JT, Fuleihan G-H, Kleerekoper M, Neer R, Peacock M, Rastad J, Silverberg SJ, Udelsman R, Wells SA. Summary statement from a workshop on asymptomatic primary hyperparathyroidism: a perspective for the 21st century. *J Clin Endocrinol Metab*. 2002;12:5353–61.
252. Auwerx J, Demedts M, Bouillon R. Altered parathyroid set point to calcium in familial hypocalciuric hypercalcaemia. *Acta Endocrinol (Copenh)*. 1984;106(2):215–8.
253. Pearce SH, Trump D, Wooding C, Besser GM, Chew SL, Grant DB, Heath DA, Hughes IA, Paterson CR, Whyte MP, et al. Calcium-sensing receptor mutations in familial benign hypercalcemia and neonatal hyperparathyroidism. *J Clin Invest*. 1995;96(6):2683–92.
254. Hendy GN, D'Souza-Li L, Yang B, Canaff L, Cole DE. Mutations of the calcium-sensing receptor (CASR) in familial hypocalciuric hypercalcemia, neonatal severe hyperparathyroidism, and autosomal dominant hypocalcemia. *Hum Mutat*. 2000;16(4):281–96.
255. Sarli M, Fradinger E, Zanchetta J. Hypocalciuric hypercalcemia due to de novo mutation of the calcium sensing receptor. *Medicina (B Aires)*. 2004;64(4):337–9.
256. Timmers HJ, Karperien M, Hamdy NA, de Boer H, Hermus AR. Normalization of serum calcium by cinacalcet in a patient with hypercalcaemia due to a de novo inactivating mutation of the calcium-sensing receptor. *J Intern Med*. 2006;260(2):177–82.
257. Attie MF, Gill Jr JR, Stock JL, Spiegel AM, Downs Jr RW, Levine MA, Marx SJ. Urinary calcium excretion in familial hypocalciuric hypercalcemia: persistence of relative hypocalciuria after induction of hypoparathyroidism. *J Clin Invest*. 1983;72:667–76.
258. Kifor O, Moore Jr FD, Delaney M, Garber J, Hendy GN, Butters R, Gao P, Cantor TL, Kifor I, Brown EM, Wysolmerski J. A syndrome of hypocalciuric

- hypercalcemia caused by autoantibodies directed at the calcium-sensing receptor. *J Clin Endocrinol Metab.* 2003;88(1):60–72.
259. Hillman DA, Scriver CR, Pedvis S, Shragovitch I. Neonatal familial primary hyperparathyroidism. *N Engl J Med.* 1964;270:483–90.
260. Waller S, Kurzawinski T, Spitz L, Thakker R, Cranston T, Pearce S, Cheetham T, van't Hoff WG. Neonatal severe hyperparathyroidism: genotype/phenotype correlation and the use of pamidronate as rescue therapy. *Eur J Pediatr.* 2004;163(10):589–94.
261. Ho C, Conner DA, Pollak MR, Ladd DJ, Kifor O, Warren HB, Brown EM, Seidman JG, Seidman CE. A mouse model of human familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. *Nat Genet.* 1995;11(4):389–94.
262. Tu Q, Pi M, Karsenty G, Simpson L, Liu S, Quarles LD. Rescue of the skeletal phenotype in *CasR*-deficient mice by transfer onto the *Gcm2* null background. *J Clin Invest.* 2003;111(7):1029–37.
263. Günther T, Chen ZF, Kim J, Priemel M, Rueger JM, Amling M, Moseley JM, Martin TJ, Anderson DJ, Karsenty G. Genetic ablation of parathyroid glands reveals another source of parathyroid hormone. *Nature.* 2000;406(6792):199–203.
264. Carling T, Udelsman R. Parathyroid surgery in familial hyperparathyroid disorders. *J Intern Med.* 2005;257:27–37.
265. Brown EM. Familial hypocalciuric hypercalcemia and other disorders with resistance to extracellular calcium. *Endocrinol Metab Clin North Am.* 2000;29:503–22.
266. Brown EM. The calcium-sensing receptor: physiology, pathophysiology and CaR-based therapeutics. *Subcell Biochem.* 2007;45:139–67.
267. Sato K, Hasegawa Y, Nakae J, Nanao K, Takahashi I, Tajima T, Shinohara N, Fujieda K. Hydrochlorothiazide effectively reduces urinary calcium excretion in two Japanese patients with gain-of-function mutations of the calcium-sensing receptor gene. *J Clin Endocrinol Metab.* 2002;87(7):3068–73.
268. Vezzoli G, Arcidiacono T, Paloschi V, Terranegra A, Biasion R, Weber G, Mora S, Syren ML, Coviello D, Cusi D, Bianchi G, Soldati L. Autosomal dominant hypocalcemia with mild type 5 Bartter syndrome. *J Nephrol.* 2006;19(4):525–8.
269. Fine KD, Santa Ana CA, Porter JL, Fordtran JS. Intestinal absorption of magnesium from food and supplements. *J Clin Invest.* 1991;2:396–402.
270. Quamme GA. Recent developments in intestinal magnesium absorption. *Curr Opin Gastroenterol.* 2008;2:230–5.
271. de Rouffignac C, Quamme G. Renal magnesium handling and its hormonal control. *Physiol Rev.* 1994; 2:305–22.
272. Quamme GA. Renal magnesium handling: new insights in understanding old problems. *Kidney Int.* 1997;5:1180–95.
273. Simon DB, Lu Y, Choate KA, Velazquez H, Al-Sabban E, Praga M, Casari G, Bettinelli A, Colussi G, Rodriguez-Soriano J, McCredie D, Milford D, Sanjad S, Lifton RP. Paracellin-1, a renal tight junction protein required for paracellular Mg^{2+} resorption. *Science.* 1999;285(5424):103–6.
274. Konrad M, Schaller A, Seelow D, Pandey AV, Waldegger S, Lesslauer A, Vitzthum H, Suzuki Y, Luk JM, Becker C, et al. Mutations in the tight-junction gene claudin 19 (*CLDN19*) are associated with renal magnesium wasting, renal failure, and severe ocular involvement. *Am J Hum Genet.* 2006;5:949–57.
275. Michelis MF, Drash AL, Linarelli LG, De Rubertis FR, Davis BB. Decreased bicarbonate threshold and renal magnesium wasting in a sibship with distal renal tubular acidosis (Evaluation of the pathophysiological role of parathyroid hormone). *Metab Clin Exp.* 1972;10:905–20.
276. Manz F, Scharer K, Janka P, Lombeck J. Renal magnesium wasting, incomplete tubular acidosis, hypercalciuria and nephrocalcinosis in sibs. *Eur J Pediatr.* 1978;2:67–79.
277. Rodriguez-Soriano J, Vallo A, Garcia-Fuentes M. Hypomagnesaemia of hereditary renal origin. *Pediatr Nephrol.* 1987;3:465–72.
278. Rodriguez-Soriano J, Vallo A. Pathophysiology of the renal acidification defect present in the syndrome of familial hypomagnesaemia-hypercalciuria. *Pediatr Nephrol.* 1994;4:431–5.
279. Praga M, Vara J, Gonzalez-Parra E, Andres A, Alamo C, Araque A, Ortiz A, Rodicio JL. Familial hypomagnesaemia with hypercalciuria and nephrocalcinosis. *Kidney Int.* 1995;5:1419–25.
280. Benigno V, Canonica CS, Bettinelli A, von Vigier RO, Truttmann AC, Bianchetti MG. Hypomagnesaemia-hypercalciuria-nephrocalcinosis: a report of nine cases and a review. *Nephrol Dial Transplant.* 2000;5:605–10.
281. Weber S, Schneider L, Peters M, Misselwitz J, Ronnefarth G, Boswald M, Bonzel KE, Seeman T, Sulakova T, Kuwertz-Broking E, et al. Novel paracellin-1 mutations in 25 families with familial hypomagnesaemia with hypercalciuria and nephrocalcinosis. *J Am Soc Nephrol.* 2001;9: 1872–81.
282. Zimmermann B, Plank C, Konrad M, Stohr W, Gravou-Apostolatos C, Rascher W, Dotsch J. Hydrochlorothiazide in *CLDN16* mutation. *Nephrol Dial Transplant.* 2006;8:2127–32.
283. Konrad M, Hou J, Weber S, Dotsch J, Kari JA, Seeman T, Kuwertz-Broking E, Peco-Antic A, Tasic V, Dittrich K, et al. *CLDN16* genotype predicts renal decline in familial hypomagnesaemia with hypercalciuria and nephrocalcinosis. *J Am Soc Nephrol.* 2008;1:171–81.
284. Godron A, Harambat J, Boccio V, et al. Familial hypomagnesaemia with hypercalciuria and nephrocalcinosis: phenotype-genotype correlation

- and outcome in 32 patients with CLDN16 or CLDN19 mutations. *Clin J Am Soc Nephrol.* 2012; 7(5):801–9.
285. Claverie-Martin F, Garcia-Nieto V, Loris C, et al. Claudin-19 mutations and clinical phenotype in Spanish patients with familial hypomagnesemia with hypercalciuria and nephrocalcinosis. *PLoS One.* 2013;8(1), e53151.
 286. Hirano T, Kobayashi N, Itoh T, Takasuga A, Nakamaru T, Hirotsune S, Sugimoto Y. Null mutation of PCLN-1/Claudin-16 results in bovine chronic interstitial nephritis. *Genome Res.* 2000;5:659–63.
 287. Ohba Y, Kitagawa H, Kitoh K, Sasaki Y, Takami M, Shinkai Y, Kunieda T. A deletion of the paracellin-1 gene is responsible for renal tubular dysplasia in cattle. *Genomics.* 2000;3:229–36.
 288. Will C, Breiderhoff T, Thumfart J, et al. Targeted deletion of murine *Cldn16* identifies extra- and intrarenal compensatory mechanisms of Ca^{2+} and Mg^{2+} wasting. *Am J Physiol Renal Physiol.* 2010;298(5):F1152–61.
 289. Blanchard A, Jeunemaitre X, Coudol P, Dechaux M, Froissart M, May A, Demontis R, Fournier A, Paillard M, Houillier P. Paracellin-1 is critical for magnesium and calcium reabsorption in the human thick ascending limb of Henle. *Kidney Int.* 2001;6:2206–15.
 290. Muller D, Kausalya PJ, Claverie-Martin F, Meij IC, Eggert P, Garcia-Nieto V, Hunziker W. A novel claudin 16 mutation associated with childhood hypercalciuria abolishes binding to ZO-1 and results in lysosomal mistargeting. *Am J Hum Genet.* 2003;6:1293–301.
 291. Hou J, Renigunta A, Konrad M, Gomes AS, Schneeberger EE, Paul DL, Waldegger S, Goodenough DA. Claudin-16 and claudin-19 interact and form a cation-selective tight junction complex. *J Clin Invest.* 2008;2:619–28.
 292. Bockenbauer D, Feather S, Stanescu HC, et al. Epilepsy, ataxia, sensorineural deafness, tubulopathy, and *KCNJ10* mutations. *N Engl J Med.* 2009; 360(19):1960–70.
 293. Scholl UI, Choi M, Liu T, et al. Seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance (SeSAME syndrome) caused by mutations in *KCNJ10*. *Proc Natl Acad Sci U S A.* 2009;106(14):5842–7.
 294. Scholl UI, Dave HB, Lu M, Farhi A, Nelson-Williams C, Listman JA, Lifton RP. SeSAME/EAST syndrome – phenotypic variability and delayed activity of the distal convoluted tubule. *Pediatr Nephrol.* 2012;27(11):2081–90.
 295. Zhang C, Wang L, Zhang J, Su XT, Lin DH, Scholl UI, Giebisch G, Lifton RP, Wang WH. *KCNJ10* determines the expression of the apical Na-Cl cotransporter (NCC) in the early distal convoluted tubule (DCT1). *Proc Natl Acad Sci U S A.* 2014;111 (32):11864–9.
 296. Neusch C, Rozengurt N, Jacobs RE, et al. Kir4.1 potassium channel subunit is crucial for oligodendrocyte development and in vivo myelination. *J Neurosci.* 2001;21(15):5429–38.
 297. Meij IC, Koenderink JB, van Bokhoven H, Assink KF, Groenestege WT, de Pont JJ, Bindels RJ, Monnens LA, van den Heuvel LP, Knoers NV. Dominant isolated renal magnesium loss is caused by misrouting of the Na(+), K(+)-ATPase gamma-subunit. *Nat Genet.* 2000;3:265–6.
 298. Geven WB, Monnens LA, Willems HL, Buijs WC, ter Haar BG. Renal magnesium wasting in two families with autosomal dominant inheritance. *Kidney Int.* 1987;5:1140–4.
 299. Meij IC, Van Den Heuvel LP, Hemmes S, Van Der Vliet WA, Willems JL, Monnens LA, Knoers NV. Exclusion of mutations in *FXYD2*, *CLDN16* and *SLC12A3* in two families with primary renal Mg(2+) loss. *Nephrol Dial Transplant.* 2003;3:512–56.
 300. Sweadner KJ, Arystarkhova E, Donnet C, Wetzel RK. *FXYD* proteins as regulators of the Na, K-ATPase in the kidney. *Ann N Y Acad Sci.* 2003;382–387.
 301. Arystarkhova E, Wetzel RK, Sweadner KJ. Distribution and oligomeric association of splice forms of Na (+)-K(+)-ATPase regulatory gamma-subunit in rat kidney. *Am J Physiol Renal Physiol.* 2002;3: F393–407.
 302. Arystarkhova E, Donnet C, Asinovski NK, Sweadner KJ. Differential regulation of renal Na, K-ATPase by splice variants of the gamma subunit. *J Biol Chem.* 2002;12:10162–72.
 303. Jones DH, Li TY, Arystarkhova E, Barr KJ, Wetzel RK, Peng J, Markham K, Sweadner KJ, Fong GH, Kidder GM. Na, K-ATPase from mice lacking the gamma subunit (*FXYD2*) exhibits altered Na+ affinity and decreased thermal stability. *J Biol Chem.* 2005;19:19003–11.
 304. Glaudemans B, van der Wijst J, Scola RH, et al. A missense mutation in the *Kv1.1* voltage-gated potassium channel-encoding gene *KCNA1* is linked to human autosomal dominant hypomagnesemia. *J Clin Invest.* 2009;119(4):936–42.
 305. van der Wijst J, Glaudemans B, Venselaar H, et al. Functional analysis of the *Kv1.1* N255D mutation associated with autosomal dominant hypomagnesemia. *J Biol Chem.* 2010;285(1):171–8.
 306. Geven WB, Monnens LA, Willems JL, Buijs W, Hamel CJ. Isolated autosomal recessive renal magnesium loss in two sisters. *Clin Genet.* 1987;6:398–402.
 307. Groenestege WM, Thebault S, van der Wijst J, van den Berg D, Janssen R, Tejpar S, van den Heuvel LP, van Cutsem E, Hoenderop JG, Knoers NV, et al. Impaired basolateral sorting of pro-EGF causes isolated recessive renal hypomagnesemia. *J Clin Invest.* 2007;8:2260–7.
 308. Paunier L, Radde IC, Kooh SW, Conen PE, Fraser D. Primary hypomagnesemia with secondary hypocalcemia in an infant. *Pediatrics.* 1968;2:385–402.
 309. Anast CS, Mohs JM, Kaplan SL, Burns TW. Evidence for parathyroid failure in magnesium deficiency. *Science.* 1972;49:606–68.

310. Shalev H, Phillip M, Galil A, Carmi R, Landau D. Clinical presentation and outcome in primary familial hypomagnesaemia. *Arch Dis Child*. 1998;2:127–30.
311. Milla PJ, Aggett PJ, Wolff OH, Harries JT. Studies in primary hypomagnesaemia: evidence for defective carrier-mediated small intestinal transport of magnesium. *Gut*. 1979;11:1028–133.
312. Matzkin H, Lotan D, Boichis H. Primary hypomagnesaemia with a probable double magnesium transport defect. *Nephron*. 1989;1:83–6.
313. Walder RY, Shalev H, Brennan TM, Carmi R, Elbedour K, Scott DA, Hanauer A, Mark AL, Patil S, Stone EM, et al. Familial hypomagnesaemia maps to chromosome 9q, not to the X chromosome: genetic linkage mapping and analysis of a balanced translocation breakpoint. *Hum Mol Genet*. 1997;9:1491–7.
314. Walder RY, Landau D, Meyer P, Shalev H, Tsolia M, Borochowitz Z, Boettger MB, Beck GE, Englehardt RK, Carmi R, et al. Mutation of TRPM6 causes familial hypomagnesaemia with secondary hypocalcaemia. *Nat Genet*. 2002;2:171–4.
315. Schlingmann KP, Sassen MC, Weber S, Pechmann U, Kusch K, Pelken L, Lotan D, Syrrou M, Prebble JJ, Cole DE, et al. Novel TRPM6 mutations in 21 families with primary hypomagnesaemia and secondary hypocalcaemia. *J Am Soc Nephrol*. 2005;10:3061–9.
316. Jalkanen R, Pronicka E, Tynymäa H, Hanauer A, Walder R, Alitalo T. Genetic background of HSH in three Polish families and a patient with an X;9 translocation. *Eur J Hum Genet*. 2006;1:55–62.
317. Guran T, Akcay T, Bereket A, et al. Clinical and molecular characterization of Turkish patients with familial hypomagnesaemia: novel mutations in TRPM6 and CLDN16 genes. *Nephrol Dial Transplant*. 2012;27(2):667–73.
318. Lainez S, Schlingmann KP, van der Wijst J, et al. New TRPM6 missense mutations linked to hypomagnesaemia with secondary hypocalcaemia. *Eur J Hum Genet*. 2013;2013.
319. Chubanov V, Schlingmann KP, Wäring J, et al. Hypomagnesaemia with secondary hypocalcaemia due to a missense mutation in the putative pore-forming region of TRPM6. *J Biol Chem*. 2007;282(10):7656–67.
320. Nadler MJ, Hermosura MC, Inabe K, Perraud AL, Zhu Q, Stokes AJ, Kurosaki T, Kinet JP, Penner R, Scharenberg AM, et al. LTRPC7 is a Mg²⁺-ATP-regulated divalent cation channel required for cell viability. *Nature*. 2001;6837:590–5.
321. Voets T, Nilius B, Hoefs S, van der Kemp AW, Droogmans G, Bindels RJ, Hoenderop JG. TRPM6 forms the Mg²⁺ influx channel involved in intestinal and renal Mg²⁺ absorption. *J Biol Chem*. 2004;279(1):19–25.
322. Cole DE, Quamme GA. Inherited disorders of renal magnesium handling. *J Am Soc Nephrol*. 2000;10:1937–47.
323. Chubanov V, Waldegger S, Mederos y Schnitzler M, Vitzthum H, Sassen MC, Seyberth HW, Konrad M, Gudermann T. Disruption of TRPM6/TRPM7 complex formation by a mutation in the TRPM6 gene causes hypomagnesaemia with secondary hypocalcaemia. *Proc Natl Acad Sci U S A*. 2004;9:2894–9.
324. Schmitz C, Dorovkov MV, Zhao X, Davenport BJ, Ryazanov AG, Perraud AL. The channel kinases TRPM6 and TRPM7 are functionally nonredundant. *J Biol Chem*. 2005;45:37763–71.
325. Groenestege WM, Hoenderup JG, van den Heuvel L, Knoers N, Bindels RJ. The epithelial Mg²⁺ channel transient receptor potential melastatin 6 is regulated by dietary Mg²⁺ contents and estrogens. *J Am Soc Nephrol*. 2006;17:1035–43.
326. Nijenhuis T, Hoenderup JG, Bindels RJ. Downregulation of Ca²⁺ and Mg²⁺ transport proteins in the kidney explains tacrolimus (FK506)-induced hypercalciuria and hypomagnesaemia. *J Am Soc Nephrol*. 2004;15:549–57.
327. Ikari A, Okude C, Sawada H, Takahashi T, Sugatani J, Miwa M. Downregulation of TRPM6-mediated magnesium influx by cyclosporine A. *Naunyn Schmiedeberg Arch Pharmacol*. 2008;377:333–43.
328. Stuijver M, Lainez S, Will C, et al. CNNM2, encoding a basolateral protein required for renal Mg²⁺ handling, is mutated in dominant hypomagnesaemia. *Am J Hum Genet*. 2011;88(3):333–43.
329. Goytain A, Quamme GA. Functional characterization of ACDP2 (ancient conserved domain protein), a divalent metal transporter. *Physiol Genomics*. 2005;22(3):382–9.
330. Meyer TE, Verwoert GC, Hwang SJ, et al. Genome-wide association studies of serum magnesium, potassium, and sodium concentrations identify six Loci influencing serum magnesium levels. *PLoS Genet*. 2010;6(8).
331. Arjona FJ, de Baaij JH, Schlingmann KP, et al. CNNM2 mutations cause impaired brain development and seizures in patients with hypomagnesaemia. *PLoS Genet*. 2014;10(4), e1004267.
332. Wang CY, Shi JD, Yang P, et al. Molecular cloning and characterization of a novel gene family of four ancient conserved domain proteins (ACDP). *Gene*. 2003;306:37–44.
333. de Baaij JH, Stuijver M, Meij IC, et al. Membrane topology and intracellular processing of cyclin M2 (CNNM2). *J Biol Chem*. 2012;287(17):13644–55.
334. Horikawa Y, Iwasaki N, Hara M, et al. Mutation in hepatocyte nuclear factor-1 beta gene (TCF2) associated with MODY. *Nat Genet*. 1997;17(4):384–5.
335. Lindner TH, Njolstad PR, Horikawa Y, et al. A novel syndrome of diabetes mellitus, renal dysfunction and genital malformation associated with a partial deletion of the pseudo-POU domain of hepatocyte nuclear factor-1beta. *Hum Mol Genet*. 1999;8(11):2001–8.
336. Faguer S, Decramer S, Chassaing N, et al. Diagnosis, management, and prognosis of HNF1B nephropathy in adulthood. *Kidney Int*. 2011;80(7):768–76.

337. Heidet L, Decramer S, Pawtowski A, et al. Spectrum of HNF1B mutations in a large cohort of patients who harbor renal diseases. *Clin J Am Soc Nephrol*. 2010; 5(6):1079–90.
338. Adalat S, Woolf AS, Johnstone KA, et al. HNF1B mutations associate with hypomagnesemia and renal magnesium wasting. *J Am Soc Nephrol*. 2009; 20(5):1123–31.
339. Ferre S, de Baaij JH, Ferreira P, et al. Mutations in PCBD1 cause hypomagnesemia and renal magnesium wasting. *J Am Soc Nephrol*. 2014;25(3): 574–86.
340. Wilson FH, Hariri A, Farhi A, Zhao H, Petersen KF, Toka HR, Nelson-Williams C, Raja KM, Kashgarian M, Shulman GI, et al. A cluster of metabolic defects caused by mutation in a mitochondrial tRNA. *Science*. 2004;269:1190–4.
341. Agus ZS. Hypomagnesemia. *J Am Soc Nephrol*. 1999;10:1616–22.
342. Schlingmann KP, Konrad M, Seyberth HW. Genetics of hereditary disorders of magnesium homeostasis. *Pediatr Nephrol*. 2004;19:13–25.
343. Gal P, Reed MD. Medications. In: Behrman RE, Kliegman R, Jenson HB, editors. *Textbook of pediatrics*. Philadelphia: Saunders; 2000. p. 2270.
344. Ranade VV, Somberg JC. Bioavailability and pharmacokinetics of magnesium after administration of magnesium salts to human. *Am J Ther*. 2001;8: 345–57.
345. Ryan MP. Magnesium and potassium-sparing diuretics. *Magnesium*. 1986;5:282–92.
346. Netzer T, Knauf H, Mutschler E. Modulation of electrolyte excretion by potassium retaining diuretics. *Eur Heart J*. 1992;13(Suppl G):22–7.
347. Bundy JT, Connito D, Mahoney MD, Pontier PJ. Treatment of idiopathic renal magnesium wasting with amiloride. *Am J Nephrol*. 1995;15:75–7.
348. Newton-Cheh C, Guo CY, Gona P, Larson MG, Benjamin EJ, Wang TJ, Kathiresan S, O'Donnell CJ, Musone SL, Camargo AL, Drake JA, Levy D, Hirschhorn JN, Vasan RS. Clinical and genetic correlates of aldosterone-to-renin ratio and relations to blood pressure in a community sample. *Hypertension*. 2007;49:846–56.
349. Vasan RS, Evans JC, Larson MG, Wilson PW, Meigs JB, Rifai N, Benjamin EJ, Levy D. Serum aldosterone and the incidence of hypertension in nonhypertensive persons. *N Engl J Med*. 2004;351:33–41.
350. Meneton P, Galan P, Bertrais S, Heudes D, Hercberg S, Menard J. High plasma aldosterone and low renin predict blood pressure increase and hypertension in middle-aged Caucasian populations. *J Hum Hypertens*. 2008;22:550–8.
351. Hannemann A, Wallaschofski H. Prevalence of primary aldosteronism in patient's cohorts and in population-based studies—a review of the current literature. *Horm Metab Res*. 2012;44:157–62.
352. Calhoun DA, Nishizaka MK, Zaman MA, Thakkar RB, Weissmann P. Hyperaldosteronism among black and white subjects with resistant hypertension. *Hypertension*. 2002;40:892–6.
353. Sutherland DJ, Ruse JL, Laidlaw JC. Hypertension, increased aldosterone secretion and low plasma renin activity relieved by dexamethasone. *Can Med Assoc J*. 1966;95:1109–19.
354. Stowasser M, Gunasekera TG, Gordon RD. Familial varieties of primary aldosteronism. *Clin Exp Pharmacol Physiol*. 2001;28:1087–90.
355. Mulatero P, di Cella SM, Williams TA, Milan A, Mengozzi G, Chiandussi L, Gomez-Sanchez CE, Veglio F. Glucocorticoid remediable aldosteronism: low morbidity and mortality in a four-generation Italian pedigree. *J Clin Endocrinol Metab*. 2002;87: 3187–91.
356. Aglony M, Martinez-Aguayo A, Carvajal CA, Campino C, Garcia H, Bancalari R, Bolte L, Avalos C, Loureiro C, Trejo P, Brinkmann K, Giadrosich V, Mericq V, Rocha A, Avila A, Perez V, Inostroza A, Fardella CE. Frequency of familial hyperaldosteronism type 1 in a hypertensive pediatric population: clinical and biochemical presentation. *Hypertension*. 2011;57:1117–21.
357. Pizzolo F, Trabetti E, Guarini P, Mulatero P, Ciacciarelli A, Blengio GS, Corrocher R, Olivieri O. Glucocorticoid remediable aldosteronism (GRA) screening in hypertensive patients from a primary care setting. *J Hum Hypertens*. 2005;19:325–7.
358. Mulatero P, Tizzani D, Viola A, Bertello C, Monticone S, Mengozzi G, Schiavone D, Williams TA, Einaudi S, La Grotta A, Rabbia F, Veglio F. Prevalence and characteristics of familial hyperaldosteronism: the PATOGEN study (Primary Aldosteronism in Torino-GENetic forms). *Hypertension*. 2011;58:797–803.
359. Litchfield WR, Anderson BF, Weiss RJ, Lifton RP, Dluhy RG. Intracranial aneurysm and hemorrhagic stroke in glucocorticoid-remediable aldosteronism. *Hypertension*. 1998;31:445–50.
360. Mantero F, Armanini D, Biazon A, Boscaro M, Carpenè G, Fallo F, Opocher G, Rocco S, Scaroni C, Sonino N. New aspects of mineralocorticoid hypertension. *Horm Res*. 1990;34:175–80.
361. Mulatero P, Veglio F, Pilon C, Rabbia F, Zocchi C, Limone P, Boscaro M, Sonino N, Fallo F. Diagnosis of glucocorticoid-remediable aldosteronism in primary aldosteronism: aldosterone response to dexamethasone and long polymerase chain reaction for chimeric gene. *J Clin Endocrinol Metab*. 1998;83: 2573–5.
362. Litchfield WR, New MI, Coolidge C, Lifton RP, Dluhy RG. Evaluation of the dexamethasone suppression test for the diagnosis of glucocorticoid-remediable aldosteronism. *J Clin Endocrinol Metab*. 1997;82:3570–3.
363. Stowasser M, Bachmann AW, Tunny TJ, Gordon RD. Production of 18-oxo-cortisol in subtypes of primary aldosteronism. *Clin Exp Pharmacol Physiol*. 1996;23:591–3.

364. Lifton RP, Dluhy RG, Powers M, Rich GM, Cook S, Ulick S, Lalouel JM. A chimaeric 11 beta-hydroxylase/aldosterone synthase gene causes glucocorticoid-remediable aldosteronism and human hypertension. *Nature*. 1992;355:262–5.
365. Curnow KM, Mulatero P, Emeric-Blanchouin N, Aupetit-Faisant B, Corvol P, Pascoe L. The amino acid substitutions Ser288Gly and Val320Ala convert the cortisol producing enzyme, CYP11B1, into an aldosterone producing enzyme. *Nat Struct Biol*. 1997;4:32–5.
366. Pascoe L, Curnow KM, Slutsker L, Connell JM, Speiser PW, New MI, White PC. Glucocorticoid-suppressible hyperaldosteronism results from hybrid genes created by unequal crossovers between CYP11B1 and CYP11B2. *Proc Natl Acad Sci U S A*. 1992;89:8327–31.
367. Jonsson JR, Klemm SA, Tunny TJ, Stowasser M, Gordon RD. A new genetic test for familial hyperaldosteronism type I aids in the detection of curable hypertension. *Biochem Biophys Res Commun*. 1995;207:565–71.
368. Funder JW, Carey RM, Fardella C, Gomez-Sanchez CE, Mantero F, Stowasser M, Young Jr WF, Montori VM. Case detection, diagnosis, and treatment of patients with primary aldosteronism: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2008;93:3266–81.
369. Stowasser M, Bachmann AW, Huggard PR, Rossetti TR, Gordon RD. Treatment of familial hyperaldosteronism type I: only partial suppression of adrenocorticotropin required to correct hypertension. *J Clin Endocrinol Metab*. 2000;85:3313–8.
370. Gordon RD, Stowasser M, Tunny TJ, Klemm SA, Finn WL, Krek AL. Clinical and pathological diversity of primary aldosteronism, including a new familial variety. *Clin Exp Pharmacol Physiol*. 1991;18:283–6.
371. Stowasser M, Gordon RD, Tunny TJ, Klemm SA, Finn WL, Krek AL. Familial hyperaldosteronism type II: five families with a new variety of primary aldosteronism. *Clin Exp Pharmacol Physiol*. 1992;19:319–22.
372. Stowasser M, Gordon RD. Primary aldosteronism: learning from the study of familial varieties. *J Hypertens*. 2000;18:1165–76.
373. Stowasser M, Gunasekera TG, Gordon RD. Familial varieties of primary aldosteronism. *Clin Exp Pharmacol Physiol*. 2001;28:1087–90.
374. Medeau V, Assie G, Zennaro MC, Clauser E, Plouin PF, Jeunemaitre X. Familial aspect of primary hyperaldosteronism: analysis of families compatible with primary hyperaldosteronism type 2. *Ann Endocrinol (Paris)*. 2005;66:240–6.
375. Lafferty AR, Torpy DJ, Stowasser M, Taymans SE, Lin JP, Huggard P, Gordon RD, Stratakis CA. A novel genetic locus for low renin hypertension: familial hyperaldosteronism type II maps to chromosome 7 (7p22). *J Med Genet*. 2000;37:831–5.
376. Sukor N, Mulatero P, Gordon RD, So A, Duffy D, Bertello C, Kelemen L, Jeske Y, Veglio F, Stowasser M. Further evidence for linkage of familial hyperaldosteronism type II at chromosome 7p22 in Italian as well as Australian and South American families. *J Hypertens*. 2008;26:1577–82.
377. So A, Jeske YW, Gordon RD, Duffy D, Kelemen L, Stowasser M. No evidence for coding region mutations in the retinoblastoma-associated Kruppel-associated box protein gene (RbA/K) causing familial hyperaldosteronism type II. *Clin Endocrinol (Oxf)*. 2006;65:829–31.
378. Jeske YW, So A, Kelemen L, Sukor N, Willys C, Bulmer B, Gordon RD, Duffy D, Stowasser M. Examination of chromosome 7p22 candidate genes RbA/K, PMS2 and GNA12 in familial hyperaldosteronism type II. *Clin Exp Pharmacol Physiol*. 2008;35:380–5.
379. Pallauf A, Schirpenbach C, Zwermann O, Fischer E, Morak M, Holinski-Feder E, Hofbauer L, Beuschlein F, Reincke M. The prevalence of familial hyperaldosteronism in apparently sporadic primary aldosteronism in Germany: a single center experience. *Horm Metab Res*. 2012;44:215–20.
380. Geller DS, Zhang JJ, Wisgerhof MV, Shackleton C, Kashgarian M, Lifton RP. A novel form of human Mendelian hypertension featuring non-glucocorticoid remediable aldosteronism. *J Clin Endocrinol Metab*. 2008;93:3117–23.
381. Choi M, Scholl UI, Yue P, Bjorklund P, Zhao B, Nelson-Williams C, Ji W, Cho Y, Patel A, Men CJ, Lolis E, Wisgerhof MV, Geller DS, Mane S, Hellman P, Westin G, Akerstrom G, Wang W, Carling T, Lifton RP. K⁺ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science*. 2011;331:768–72.
382. Oki K, Plonczynski MW, Luis Lam M, Gomez-Sanchez EP, Gomez-Sanchez CE. Potassium channel mutant KCNJ5 T158A expression in HAC-15 cells increases aldosterone synthesis. *Endocrinology*. 2012;153:1774–82.
383. Scholl UI, Nelson-Williams C, Yue P, Grekin R, Wyatt RJ, Dillon MJ, Couch R, Hammer LK, Harley FL, Farhi A, Wang WH, Lifton RP. Hypertension with or without adrenal hyperplasia due to different inherited mutations in the potassium channel KCNJ5. *Proc Natl Acad Sci U S A*. 2012;109:2533–8.
384. Charmandari E, Sertedaki A, Kino T, Merakou C, Hoffman DA, Hatch MM, Hurt DE, Lin L, Xekouki P, Stratakis CA, Chrousos GP. A novel point mutation in the KCNJ5 gene causing primary hyperaldosteronism and early-onset autosomal dominant hypertension. *J Clin Endocrinol Metab*. 2012;97:E1532–9.
385. Mulatero P, Tauber P, Zennaro MC, Monticone S, Lang K, Beuschlein F, Fischer E, Tizzani D, Pallauf A, Viola A, Amar L, Williams TA, Strom TM, Graf E, Bandulik S, Penton D, Plouin PF, Warth R, Allolio B, Jeunemaitre X, Veglio F,

- Reincke M. KCNJ5 mutations in European families with nonglucocorticoid remediable familial hyperaldosteronism. *Hypertension*. 2012;59:235–40.
386. Monticone S, Hattangady NG, Penton D, Isaacs CM, Edwards MA, Williams TA, Sterner C, Warth R, Mulatero P, Rainey WE. A novel Y152C KCNJ5 mutation responsible for familial hyperaldosteronism type III. *J Clin Endocrinol Metab*. 2013;98:E1861–5.
387. Adachi M, Muroya K, Asakura Y, Sugiyama K, Homma K, Hasegawa T. Discordant genotype-phenotype correlation in familial hyperaldosteronism type III with KCNJ5 gene mutation: a patient report and review of the literature. *Horm Res Pediatr*. 2014;82:138–42.
388. Liddle G, Bledsoe T, Coppage WS. A familial renal disorder simulating primary aldosteronism but with negligible aldosterone secretion. *Trans Assoc Am Physicians*. 1963;76:199–213.
389. Botero-Velez M, Curtis JJ, Warnock DG. Brief report: Liddle's syndrome revisited – a disorder of sodium reabsorption in the distal tubule. *N Engl J Med*. 1994;330:178–81.
390. Shimkets RA, Warnock DG, Bositis CM, Nelson-Williams C, Hansson JH, Schambelan M, Gill JR, Ulick S, Milora RV, Findling JW, et al. Liddle's syndrome: heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel. *Cell*. 1994;79:407–14.
391. Jeunemaitre X, Bassilana F, Persu A, Dumont C, Champigny G, Lazdunski M, Corvol P, Barbry P. Genotype-phenotype analysis of a newly discovered family with Liddle's syndrome. *J Hypertens*. 1997;15:1091–100.
392. Baker E, Jeunemaitre X, Portal AJ, Grimbert P, Markandu N, Persu A, Corvol P, MacGregor G. Abnormalities of nasal potential difference measurement in Liddle's syndrome. *J Clin Invest*. 1998;102:10–4.
393. Hansson JH, Nelson-Williams C, Suzuki H, Schild L, Shimkets R, Lu Y, Canessa C, Iwasaki T, Rossier B, Lifton RP. Hypertension caused by a truncated epithelial sodium channel gamma subunit: genetic heterogeneity of Liddle syndrome. *Nat Genet*. 1995;11:76–82.
394. Yang KQ, Lu CX, Xiao Y, Liu YX, Jiang XJ, Zhang X, Zhou XL. Molecular genetics of Liddle's syndrome. *Clin Endocrinol (Oxf)*. 2014;82(4):611–4.
395. Canessa CM, Schild L, Buell G, Thorens B, Gautschi I, Horisberger JD, Rossier BC. Amiloride-sensitive epithelial Na⁺ channel is made of three homologous subunits. *Nature*. 1994;367:463–7.
396. Jasti J, Furukawa H, Gonzales EB, Gouaux E. Structure of acid-sensing ion channel 1 at 1.9 Å resolution and low pH. *Nature*. 2007;449:316–23.
397. Rossier BC. Epithelial sodium channel (ENaC) and the control of blood pressure. *Curr Opin Pharmacol*. 2014;15:33–46.
398. Snyder PM, Price MP, McDonald FJ, Adams CM, Volk KA, Zeiher BG, Stokes JB, Welsh MJ. Mechanism by which Liddle's syndrome mutations increase activity of a human epithelial Na⁺ channel. *Cell*. 1995;83:969–78.
399. Schild L, Lu Y, Gautschi I, Schneeberger E, Lifton RP, Rossier BC. Identification of a PY motif in the epithelial Na channel subunits as a target sequence for mutations causing channel activation found in Liddle syndrome. *EMBO J*. 1996;15:2381–7.
400. Rotin D, Staub O, Haguenaer-Tsapis R. Ubiquitination and endocytosis of plasma membrane proteins: role of Nedd4/Rsp5p family of ubiquitin-protein ligases. *J Membr Biol*. 2000;176:1–17.
401. Staub O, Verrey F. Impact of Nedd4 proteins and serum and glucocorticoid-induced kinases on epithelial Na⁺ transport in the distal nephron. *J Am Soc Nephrol*. 2005;16:3167–74.
402. Firsov D, Schild L, Gautschi I, Merillat AM, Schneeberger E, Rossier BC. Cell surface expression of the epithelial Na channel and a mutant causing Liddle syndrome: a quantitative approach. *Proc Natl Acad Sci U S A*. 1996;93:15370–5.
403. Warnock DG. Liddle syndrome: an autosomal dominant form of human hypertension. *Kidney Int*. 1998;53(1):18–24.
404. Teiwes J, Toto RD. Epithelial sodium channel inhibition in cardiovascular disease. A potential role for amiloride. *Am J Hypertens*. 2007;20:109–17.
405. Swift PA, MacGregor GA. The epithelial sodium channel in hypertension: genetic heterogeneity and implications for treatment with amiloride. *Am J Pharmacogenomics*. 2004;4:161–8.
406. Geller DS, Farhi A, Pinkerton N, Fradley M, Moritz M, Spitzer A, Meinke G, Tsai FT, Sigler PB, Lifton RP. Activating mineralocorticoid receptor mutation in hypertension exacerbated by pregnancy. *Science*. 2000;289:119–23.
407. Rafestin-Oblin ME, Souque A, Bocchi B, Pinon G, Fagart J, Vandewalle A. The severe form of hypertension caused by the activating S810L mutation in the mineralocorticoid receptor is cortisone related. *Endocrinology*. 2003;144:528–33.
408. New MI, Levine LS, Biglieri EG, Pareira J, Ulick S. Evidence for an unidentified steroid in a child with apparent mineralocorticoid hypertension. *J Clin Endocrinol Metab*. 1977;44:924–33.
409. Ulick S, Ramirez LC, New MI. An abnormality in steroid reductive metabolism in a hypertensive syndrome. *J Clin Endocrinol Metab*. 1977;44:799–802.
410. Stewart PM. Mineralocorticoid hypertension. *Lancet*. 1999;353:1341–7.
411. Ulick S, Tedde R, Mantero F. Pathogenesis of the type 2 variant of the syndrome of apparent mineralocorticoid excess. *J Clin Endocrinol Metab*. 1990;70:200–6.
412. Li A, Tedde R, Krozowski ZS, Pala A, Li KX, Shackleton CH, Mantero F, Palermo M, Stewart PM. Molecular basis for hypertension in the “type II variant” of apparent mineralocorticoid excess. *Am J Hum Genet*. 1998;63:370–9.

413. Edwards CR, Stewart PM, Burt D, Brett L, McIntyre MA, Sutanto WS, de Kloet ER, Monder C. Localisation of 11 beta-hydroxysteroid dehydrogenase – tissue specific protector of the mineralocorticoid receptor. *Lancet*. 1988;2:986–9.
414. Mune T, Rogerson FM, Nikkila H, Agarwal AK, White PC. Human hypertension caused by mutations in the kidney isozyme of 11 beta-hydroxysteroid dehydrogenase. *Nat Genet*. 1995;10:394–9.
415. Stewart PM, Krozowski ZS, Gupta A, Milford DV, Howie AJ, Sheppard MC, Whorwood CB. Hypertension in the syndrome of apparent mineralocorticoid excess due to mutation of the 11 beta-hydroxysteroid dehydrogenase type 2 gene. *Lancet*. 1996;347:88–91.
416. Morineau G, Marc JM, Boudi A, Galons H, Gourmelin M, Corvol P, Pascoe L, Fiet J. Genetic, biochemical, and clinical studies of patients with A328V or R213C mutations in 11betaHSD2 causing apparent mineralocorticoid excess. *Hypertension*. 1999;34:435–41.
417. New MI, Geller DS, Fallo F, Wilson RC. Monogenic low renin hypertension. *Trends Endocrinol Metab*. 2005;16:92–7.
418. Kotelevtsev Y, Brown RW, Fleming S, Kenyon C, Edwards CR, Seckl JR, Mullins JJ. Hypertension in mice lacking 11beta-hydroxysteroid dehydrogenase type 2. *J Clin Invest*. 1999;103:683–9.
419. Gourmelin M, Saint-Jacques I, Morineau G, Soliman H, Julien R, Fiet J. 11 beta-Hydroxysteroid dehydrogenase deficit: a rare cause of arterial hypertension. Diagnosis and therapeutic approach in two young brothers. *Eur J Endocrinol*. 1996;135:238–44.
420. Palermo M, Delitala G, Sorba G, Cossu M, Satta R, Tedde R, Pala A, Shackleton CH. Does kidney transplantation normalise cortisol metabolism in apparent mineralocorticoid excess syndrome? *J Endocrinol Invest*. 2000;23:457–62.
421. Wilson RC, Nimkarn S, New MI. Apparent mineralocorticoid excess. *Trends Endocrinol Metab*. 2001;12:104–11.
422. Gordon RD, Klemm SA, Tunny TJ, Stowasser M. Chapter 125: Gordon syndrome: a sodium-volume-dependent form of hypertension with a genetic basis. In: Laragh JH, Brenner BM, editors. *Hypertension: pathophysiology, diagnosis and management*. 2nd ed. New York: Raven; 1995.
423. Achard JM, Disse-Nicodeme S, Fiquet-Kempf B, Jeunemaitre X. Phenotypic and genetic heterogeneity of familial hyperkalaemic hypertension (Gordon syndrome). *Clin Exp Pharmacol Physiol*. 2001;28:1048–52.
424. Disse-Nicodeme S, Achard JM, Desitter I, Houot AM, Fournier A, Corvol P, Jeunemaitre X. A new locus on chromosome 12p13.3 for pseudohypoaldosteronism type II, an autosomal dominant form of hypertension. *Am J Hum Genet*. 2000;67:302–10.
425. Wilson FH, Disse-Nicodeme S, Choate KA, Ishikawa K, Nelson-Williams C, Desitter I, Gunel M, Milford DV, Lipkin GW, Achard JM, Feely MP, Dussol B, Berland Y, Unwin RJ, Mayan H, Simon DB, Farfel Z, Jeunemaitre X, Lifton RP. Human hypertension caused by mutations in WNK kinases. *Science*. 2001;293:1107–12.
426. Xu BE, Lee BH, Min X, Lenertz L, Heise CJ, Stippec S, Goldsmith EJ, Cobb MH. WNK1: analysis of protein kinase structure, downstream targets, and potential roles in hypertension. *Cell Res*. 2005;15:6–10.
427. Vidal-Petiot E, Elvira-Matlot E, Mutig K, Soukaseum C, Baudrie V, Wu S, Cheval L, Huc E, Cambillau M, Bachmann S, Doucet A, Jeunemaitre X, Hadchouel J. WNK1-related familial hyperkalaemic hypertension results from an increased expression of L-WNK1 specifically in the distal nephron. *Proc Natl Acad Sci U S A*. 2013;110(35):14366–71.
428. Boyden LM, Choi M, Choate KA, Nelson-Williams CJ, Farhi A, Toka HR, Tikhonova IR, Bjornson R, Mane SM, Colussi G, Lebel M, Gordon RD, Semmekrot BA, Poujol A, Valimaki MJ, De Ferrari ME, Sanjad SA, Gutkin M, Karet FE, Tucci JR, Stockigt JR, Keppler-Noreuil KM, Porter CC, Anand SK, Whiteford ML, Davis ID, Dewar SB, Bettinelli A, Fadrowski JJ, Belsha CW, Hunley TE, Nelson RD, Trachtman H, Cole TR, Pinsky M, Bockenhauer D, Shenoy M, Vaidyanathan P, Foreman JW, Rasoulopour M, Thameem F, Al-Shahroui HZ, Radhakrishnan J, Gharavi AG, Goilav B, Lifton RP. Mutations in kelch-like 3 and cullin 3 cause hypertension and electrolyte abnormalities. *Nature*. 2012;482:98–102.
429. Louis-Dit-Picard H, Barc J, Trujillano D, Miserey-Lenkei S, Bouatia-Naji N, Pylypenko O, Beaurain G, Bonnefond A, Sand O, Simian C, Vidal-Petiot E, Soukaseum C, Mandet C, Broux F, Chabre O, Delahousse M, Esnault V, Fiquet B, Houillier P, Bagnis CI, Koenig J, Konrad M, Landais P, Mourani C, Niaudet P, Probst V, Thauvin C, Unwin RJ, Soroka SD, Ehret G, Ossowski S, Caulfield M, International Consortium for Blood P, Bruneval P, Estivill X, Froguel P, Hadchouel J, Schott JJ, Jeunemaitre X. KLHL3 mutations cause familial hyperkalaemic hypertension by impairing ion transport in the distal nephron. *Nat Genet*. 2012;44(456–460):S451–3.
430. Schumacher FR, Sorrell FJ, Alessi DR, Bullock AN, Kurz T. Structural and biochemical characterization of the KLHL3-WNK kinase interaction important in blood pressure regulation. *Biochem J*. 2014;460:237–46.
431. Kahle KT, Wilson FH, Leng Q, Lalioti MD, O'Connell AD, Dong K, Rapson AK, MacGregor GG, Giebisch G, Hebert SC, Lifton RP. WNK4 regulates the balance between renal NaCl reabsorption and K⁺ secretion. *Nat Genet*. 2003;35:372–6.
432. Hadchouel J, Delaloy C, Faure S, Achard JM, Jeunemaitre X. Familial hyperkalaemic hypertension. *J Am Soc Nephrol*. 2006;17:208–17.

433. Hoom EJ, Nelson JH, McCormick JA, Ellison DH. The WNK kinase network regulating sodium, potassium, and blood pressure. *J Am Soc Nephrol*. 2011;22:605–14.
434. Uchida S. Regulation of blood pressure and renal electrolyte balance by Cullin-RING ligases. *Curr Opin Nephrol Hypertens*. 2014;23:487–93.
435. Chaves-Canales M, Zhang C, Soukaseum C, Moreno E, Pacheco-Alvarez D, Vidal-Petiot E, Castañeda-Bueno M, Vazquez N, Rojas-Vega L, Meermeier NP, Rogers S, Jeunemaitre X, Yang CL, Ellison DH, Gamba G, Hadchouel J. The WNK-SPAK-NCC cascade revisited: WNK1 stimulates the activity of the NaCl cotransporter via SPAK, an effect antagonized by WNK4. *Hypertension*. 2014;64:1047–53.
436. Lalioti MD, Zhang J, Volkman HM, Kahle KT, Hoffmann KE, Toka HR, Nelson-Williams C, Ellison DH, Flavell R, Booth CJ, Lu Y, Geller DS, Lifton RP. Wnk4 controls blood pressure and potassium homeostasis via regulation of mass and activity of the distal convoluted tubule. *Nat Genet*. 2006;38:1124–32.
437. San-Cristobal P, Pacheco-Alvarez D, Richardson C, Ring AM, Vazquez N, Rafiqi FH, Chari D, Kahle KT, Leng Q, Bobadilla NA, Hebert SC, Alessi DR, Lifton RP, Gamba G. Angiotensin II signaling increases activity of the renal Na-Cl cotransporter through a WNK4-SPAK-dependent pathway. *Proc Natl Acad Sci U S A*. 2009;106:4384–9.
438. Ohta A, Schumacher FR, Mehellou Y, Johnson C, Knebel A, Macartney TJ, Wood NT, Alessi DR, Kurz T. The CUL3-KLHL3 E3 ligase complex mutated in Gordon's hypertension syndrome interacts with and ubiquitylates WNK isoforms: disease-causing mutations in KLHL3 and WNK4 disrupt interaction. *Biochem J*. 2013;451:111–22.
439. Wakabayashi M, Mori T, Isobe K, Sohara E, Susa K, Araki Y, Chiga M, Kikuchi E, Nomura N, Mori Y, Matsuo H, Murata T, Nomura S, Asano T, Kawaguchi H, Nonoyama S, Rai T, Sasaki S, Uchida S. Impaired KLHL3-mediated ubiquitination of WNK4 causes human hypertension. *Cell Rep*. 2013;3:858–68.
440. Delaloy C, Lu J, Houot AM, Disse-Nicodeme S, Gasc JM, Corvol P, Jeunemaitre X. Multiple promoters in the WNK1 gene: one controls expression of a kidney-specific kinase-defective isoform. *Mol Cell Biol*. 2003;23:9208–21.
441. Xu BE, Min X, Stippec S, Lee BH, Goldsmith EJ, Cobb MH. Regulation of WNK1 by an autoinhibitory domain and autophosphorylation. *J Biol Chem*. 2002;277:48456–62.
442. Píala AT, Moon TM, Akella R, He H, Cobb MH, Goldsmith EJ. Chloride sensing by WNK1 involves inhibition of autophosphorylation. *Sci Signal*. 2014;7:ra41.
443. Bazúa-Valenti S, Chávez-Canales M, Rojas-Vega L, González-Rodríguez X, Vázquez N, Rodríguez-Gama A, Argaiz ER, Melo Z, Plata C, Ellison DH, García-Valdés J, Hadchouel J, Gamba G. The effect of WNK4 on the Na⁺-Cl⁻ cotransporter is modulated by intracellular chloride. *J Am Soc Nephrol*. 2015;26(8):1781–6.
444. Achard JM, Warnock DG, Disse-Nicodeme S, Fiquet-Kempf B, Corvol P, Fournier A, Jeunemaitre X. Familial hyperkalemic hypertension: phenotypic analysis in a large family with the WNK1 deletion mutation. *Am J Med*. 2003;114:495–8.
445. Mayan H, Gurevitz O, Farfel Z. Successful treatment by cyclooxygenase-2 inhibitor of refractory hypokalemia in a patient with Gitelman's syndrome. *Clin Nephrol*. 2002;58:73–6.
446. Sanjad S, Mansour F, Hernandez R, Hill L. Severe hypertension, hyperkalemia, and renal tubular acidosis responding to dietary sodium restriction. *Pediatrics*. 1982;69:317–24.
447. Glover M, O'Shaughnessy KM. SPAK and WNK kinases: a new target for blood pressure treatment? *Curr Opin Nephrol Hypertens*. 2011;20:16–22.
448. Zennaro MC, Hubert EL, Fernandes-Rosa FL. Aldosterone resistance: structural and functional considerations and new perspectives. *Mol Cell Endocrinol*. 2012;350:206–15.
449. Cheek DB, Perry JW. A salt wasting syndrome in infancy. *Arch Dis Child*. 1958;33:252–6.
450. Hanukoglu A. Type I pseudohypoaldosteronism includes two clinically and genetically distinct entities with either renal or multiple target organ defects. *J Clin Endocrinol Metab*. 1991;73:936–44.
451. Geller DS. Mineralocorticoid resistance. *Clin Endocrinol (Oxf)*. 2005;62:513–20.
452. Zettle RM, West ML, Josse RG, Richardson RM, Marsden PA, Halperin ML. Renal potassium handling during states of low aldosterone bio-activity: a method to differentiate renal and non-renal causes. *Am J Nephrol*. 1987;7:360–6.
453. Rodriguez-Soriano J, Ubetagoyena M, Vallo A. Transtubular potassium concentration gradient: a useful test to estimate renal aldosterone bio-activity in infants and children. *Pediatr Nephrol*. 1990;4:105–10.
454. Escoubet B, Couffignal C, Laisy JP, Mangin L, Chillon S, Laouenan C, Serfaty JM, Jeunemaitre X, Mentre F, Zennaro MC. Cardiovascular effects of aldosterone: insight from adult carriers of mineralocorticoid receptor mutations. *Circ Cardiovasc Genet*. 2013;6:381–90.
455. Oberfield SE, Levine LS, Carey RM, Bejar R, New MI. Pseudohypoaldosteronism: multiple target organ unresponsiveness to mineralocorticoid hormones. *J Clin Endocrinol Metab*. 1979;48:228–34.
456. Wong GP, Levine D. Congenital pseudohypoaldosteronism presenting in utero with acute polyhydramnios. *J Matern Fetal Med*. 1998;7:76–8.
457. Speiser PW, Stoner E, New MI. Pseudohypoaldosteronism: a review and report of two new cases. *Adv Exp Med Biol*. 1986;196:173–95.

458. Kerem E, Bistrizter T, Hanukoglu A, Hofmann T, Zhou Z, Bennett W, MacLaughlin E, Barker P, Nash M, Quittell L, Boucher R, Knowles MR. Pulmonary epithelial sodium-channel dysfunction and excess airway liquid in pseudohypoaldosteronism. *N Engl J Med*. 1999;341:156–62.
459. Martin JM, Caldich L, Monteagudo C, Alonso V, Garcia L, Jorda E. Clinico-pathological analysis of the cutaneous lesions of a patient with type I pseudohypoaldosteronism. *J Eur Acad Dermatol Venereol*. 2005;19:377–9.
460. Belot A, Ranchin B, Fichtner C, Pujo L, Rossier BC, Liutkus A, Morlat C, Nicolino M, Zennaro MC, Cochat P. Pseudohypoaldosteronisms, report on a 10-patient series. *Nephrol Dial Transplant*. 2008;23:1636–41.
461. Rodriguez-Soriano J, Vallo A, Oliveros R, Castillo G. Transient pseudohypoaldosteronism secondary to obstructive uropathy in infancy. *J Pediatr*. 1983;103:375–80.
462. Bulchmann G, Schuster T, Heger A, Kuhnle U, Joppich I, Schmidt H. Transient pseudohypoaldosteronism secondary to posterior urethral valves – a case report and review of the literature. *Eur J Pediatr Surg*. 2001;11:277–9.
463. Watanabe T, Nitta K. Transient hyporeninemic hypoaldosteronism in acute glomerulonephritis. *Pediatr Nephrol*. 2002;17:959–63.
464. Vantghem MC, Hober C, Evrard A, Ghulam A, Lescut D, Racadot A, Triboulet JP, Armanini D, Lefebvre J. Transient pseudo-hypoaldosteronism following resection of the ileum: normal level of lymphocytic aldosterone receptors outside the acute phase. *J Endocrinol Invest*. 1999;22:122–7.
465. Deppe CE, Heering PJ, Viengchareun S, Grabensee B, Farman N, Lombes M. Cyclosporine a and FK506 inhibit transcriptional activity of the human mineralocorticoid receptor: a cell-based model to investigate partial aldosterone resistance in kidney transplantation. *Endocrinology*. 2002;143:1932–41.
466. Verrey F, Pearce D, Pfeiffer R, Spindler B, Mastroberardino L, Summa V, Zecevic M. Pleiotropic action of aldosterone in epithelia mediated by transcription and post-transcription mechanisms. *Kidney Int*. 2000;57:1277–82.
467. Pascual-Le Tallec L, Lombes M. The mineralocorticoid receptor: a journey exploring its diversity and specificity of action. *Mol Endocrinol*. 2005;19:2211–21; Stockand JD (2002) New ideas about aldosterone signaling in epithelia. *Am J Physiol Renal Physiol* 282:F559–576.
468. Armanini D, Kuhnle U, Strasser T, Dorr H, Butenandt I, Weber P, Stockigt JR, Pearce P, Funder JW. Aldosterone receptor deficiency in pseudohypoaldosteronism. *N Engl J Med*. 1985;313:1178–81.
469. Kuhnle U, Nielsen MD, Tietze HU, Schroeter CH, Schlamp D, Bosson D, Knorr D, Armanini D. Pseudohypoaldosteronism in eight families: different forms of inheritance are evidence for various genetic defects. *J Clin Endocrinol Metab*. 1990;70:638–41.
470. Geller DS, Rodriguez-Soriano J, Vallo Boado A, Schifter S, Bayer M, Chang SS, Lifton RP. Mutations in the mineralocorticoid receptor gene cause autosomal dominant pseudohypoaldosteronism type I. *Nat Genet*. 1998;19:279–81.
471. Riepe FG, Finkeldei J, de Sanctis L, Einaudi S, Testa A, Karges B, Peter M, Viemann M, Grotzinger J, Sippell WG, Fejes-Toth G, Krone N. Elucidating the underlying molecular pathogenesis of NR3C2 mutants causing autosomal dominant pseudohypoaldosteronism type 1. *J Clin Endocrinol Metab*. 2006;91:4552–61.
472. Pujo L, Fagart J, Gary F, Papadimitriou DT, Claes A, Jeunemaitre X, Zennaro MC. Mineralocorticoid receptor mutations are the principal cause of renal type 1 pseudohypoaldosteronism. *Hum Mutat*. 2007;28:33–40.
473. Zennaro MC, Keightley MC, Kotelevtsev Y, Conway GS, Soubrier F, Fuller PJ. Human mineralocorticoid receptor genomic structure and identification of expressed isoforms. *J Biol Chem*. 1995;270:21016–20.
474. Sartorato P, Lapeyraque AL, Armanini D, Kuhnle U, Khaldi Y, Salomon R, Abadie V, Di Battista E, Naselli A, Racine A, Bosio M, Caprio M, Poulet-Young V, Chabrolle JP, Niaudet P, De Gennes C, Lecornec MH, Poisson E, Fusco AM, Loli P, Lombes M, Zennaro MC. Different inactivating mutations of the mineralocorticoid receptor in fourteen families affected by type I pseudohypoaldosteronism. *J Clin Endocrinol Metab*. 2003;88:2508–17.
475. Geller DS, Zhang J, Zennaro MC, Vallo-Boado A, Rodriguez-Soriano J, Furu L, Haws R, Metzger D, Botelho B, Karaviti L, Haqq AM, Corey H, Janssens S, Corvol P, Lifton RP. Autosomal dominant pseudohypoaldosteronism type 1: mechanisms, evidence for neonatal lethality, and phenotypic expression in adults. *J Am Soc Nephrol*. 2006;17:1429–36.
476. Sartorato P, Khaldi Y, Lapeyraque AL, Armanini D, Kuhnle U, Salomon R, Caprio M, Viengchareun S, Lombes M, Zennaro MC. Inactivating mutations of the mineralocorticoid receptor in type I pseudohypoaldosteronism. *Mol Cell Endocrinol*. 2004;217:119–25.
477. Fernandes-Rosa FL, Hubert EL, Fagart J, Tchitchek N, Gomes D, Jouanno E, Benecke A, Rafestin-Oblin ME, Jeunemaitre X, Antonini SR, Zennaro MC. Mineralocorticoid receptor mutations differentially affect individual gene expression profiles in pseudohypoaldosteronism type 1. *J Clin Endocrinol Metab*. 2011;96:E519–27.
478. Rossier BC, Baker ME, Studer RA. Epithelial sodium transport and its control by aldosterone: the story of our internal environment revisited. *Physiol Rev*. 2015;95:297–340.
479. Chang SS, Grunder S, Hanukoglu A, Rosler A, Mathew PM, Hanukoglu I, Schild L, Lu Y, Shimkets RA, Nelson-Williams C, Rossier BC, Lifton RP.

- Mutations in subunits of the epithelial sodium channel cause salt wasting with hyperkalaemic acidosis, pseudohypoaldosteronism type 1. *Nat Genet.* 1996;12:248–53.
480. Strautnieks SS, Thompson RJ, Gardiner RM, Chung E. A novel splice-site mutation in the gamma subunit of the epithelial sodium channel gene in three pseudohypoaldosteronism type 1 families. *Nat Genet.* 1996;13:248–50.
481. Rossier BC, Pradervand S, Schild L, Hummler E. Epithelial sodium channel and the control of sodium balance: interaction between genetic and environmental factors. *Annu Rev Physiol.* 2002;64:877–97.
482. Hanukoglu A, Edelheit O, Shriki Y, Gizewska M, Dascal N, Hanukoglu I. Renin-aldosterone response, urinary Na/K ratio and growth in pseudohypoaldosteronism patients with mutations in epithelial sodium channel (ENaC) subunit genes. *J Steroid Biochem Mol Biol.* 2008;111:268–74.
483. Edelheit O, Hanukoglu I, Shriki Y, Tfilin M, Dascal N, Gillis D, Hanukoglu A. Truncated beta epithelial sodium channel (ENaC) subunits responsible for multi-system pseudohypoaldosteronism support partial activity of ENaC. *J Steroid Biochem Mol Biol.* 2010;119:84–8.
484. Riepe FG, van Bemmelen MX, Cachat F, Plendl H, Gautschi I, Krone N, Holterhus PM, Theintz G, Schild L. Revealing a subclinical salt-losing phenotype in heterozygous carriers of the novel S562P mutation in the alpha subunit of the epithelial sodium channel. *Clin Endocrinol.* 2009;70:252–8.
485. Dirlwanger M, Huser D, Zennaro MC, Girardin E, Schild L, Schwitzgebel VM. A homozygous missense mutation in SCNN1A is responsible for a transient neonatal form of pseudohypoaldosteronism type 1. *Am J Physiol Endocrinol Metab.* 2011;301:E467–73.
486. Hubert EL, Teissier R, Fernandes-Rosa FL, Fay M, Rafestin-Oblin ME, Jeunemaitre X, Metz C, Escoubet B, Zennaro MC. Mineralocorticoid receptor mutations and a severe recessive pseudohypoaldosteronism type 1. *J Am Soc Nephrol.* 2011;22:1997–2003.
487. New MI. Inborn errors of adrenal steroidogenesis. *Mol Cell Endocrinol.* 2003;211:75–83.
488. Finer G, Shalev H, Birk OS, Galron D, Jeck N, Sinai-Treiman L, Landau D. Transient neonatal hyperkalemia in the antenatal (ROMK defective) Bartter syndrome. *J Pediatr.* 2003;142:318–23.
489. Loomba-Albrecht LA, Nagel M, Bremer AA. Pseudohypoaldosteronism type 1 due to a novel mutation in the mineralocorticoid receptor gene. *Horm Res Paediatr.* 2010;73:482–6.
490. Mathew PM, Manasra KB, Hamdan JA. Indomethacin and cation-exchange resin in the management of pseudohypoaldosteronism. *Clin Pediatr.* 1993;32:58–60.
491. Hanukoglu A, Hanukoglu I. Clinical improvement in patients with autosomal recessive pseudohypoaldosteronism and the necessity for salt supplementation. *Clin Exp Nephrol.* 2010;14:518–9.