38 Tubular Disorders of Electrolyte Regulation

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In this section, we will discuss the inherited disorders associated with defective tubular handling of NaCl, causing secondary aldosteronism and hypokalemia (Bartter-like syndromes), abnormal handling of calcium and magnesium, the states of low-renin hypertension with hypokalemia, and the two forms of pseudohypoaldosteronism (type I and type II). We will not address other types of inherited tubulopathies, such as renal Fanconi syndrome, diabetes insipidus, or renal tubular acidosis, which are detailed elsewhere.

Bartter-Like Syndromes

Introduction

In 1962, F. Bartter and co-workers described two African American patients with a new syndrome, 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 relative vascular 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 markedly differ in terms of age of onset, severity of symptoms, presence of urinary concentrating defect, other electrolyte abnormalities (including 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 Na^+–K^+–$2Cl^-$ 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 recycles K^+ across the luminal membrane, whereas the ClC-Ka and ClC-Kb Cl^- channels coupled to their beta-subunit barttin are responsible for the basolateral exit of Cl^- (*Fig. 38-1*). These concerted transport processes are crucial for the vectorial NaCl transport in the TAL, and thus the urinary concentrating ability, and for generating the lumen-positive electrical charge that drives the paracellular reabsorption of 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 ClC-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 hypocalciuria.

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

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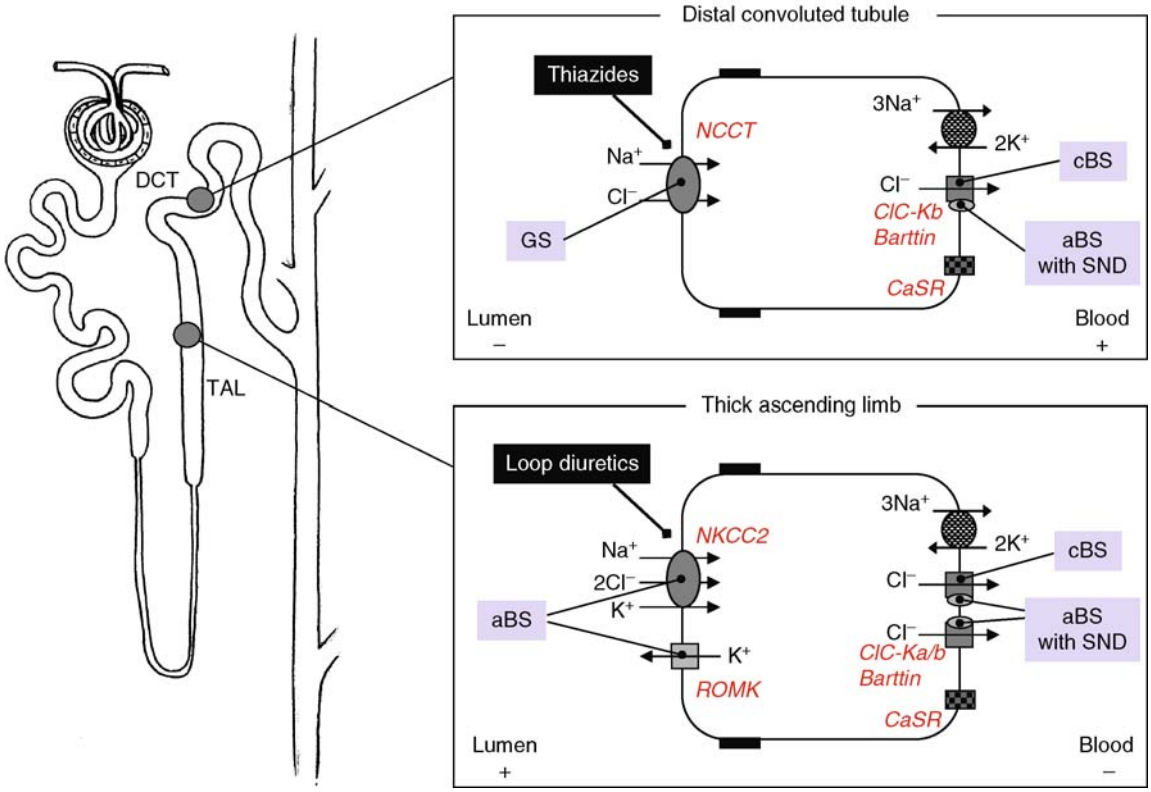
Figure 38-1

Molecular basis of Bartter-like syndromes. Approximately 25% of the filtered NaCl is reabsorbed in the thick ascending limb (TAL) via the apical Na^+–K^+–$2Cl^-$ 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 Na^+–K^+-ATPase and the Cl^- channels ClC–Ka and ClC–Kb associated with the regulatory beta-subunit Barttin mediate the exit of Na^+ and Cl^- ions from the cells. The thiazide-sensitive Na^+–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 beta-subunit barttin cause antenatal Bartter syndrome with sensorineural deafness (aBS with SND) and mutations in *CLCNKB* (ClC-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.



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, a series of 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 encoding the apical NKCC2 (6) or ROMK (7), whereas inactivating mutations in barttin, a regulatory beta-subunit of the basolateral ClC-Ka and ClC-Kb channels, were detected in aBS with SND (8). On the other hand, inactivating mutations of ClC-Kb, which is located both in the TAL and DCT, were associated with the cBS (9), whereas GS was found to be associated with mutations of NCCT (10) (*Fig. 38-1*).

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 38-1*). When discussing such patients, the clinical diagnosis of the BS subtype, based on relatively simple clinical criteria, should

Table 38-1

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	*SLC12A1*	Na^+–K^+–$2Cl^-$ cotransporter NKCC2	TAL
Antenatal Bartter syndrome (aBS), Hyperprostaglandin-E syndrome (HPS), Type II Bartter syndrome[a]	241200	AR	11q24	*KCNJ1*	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	*BSND*	Barttin, beta-subunit of ClC-Ka/b	TAL + DCT
Classic Bartter syndrome (cBS) Type III Bartter syndrome[a]	607364	AR	1p36	*CLCNKB*	Cl^- channel ClC-Kb	TAL + DCT
Gitelman syndrome (GS)	263800	AR	16q13	*SLC12A3*	Na^+-Cl^- cotransporter NCCT	DCT

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

[a]This classification is based on the chronological order of gene discovery

[b]A digenic disorder with inactivating mutations of *CLCNKA* and *CLCNKB* has been associated with the aBS with SND phenotype

be completed whenever possible by the genotyping information since there is no direct genotype-phenotype correlation in these diseases (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* with the development of polyhydramnios and premature delivery in almost all cases. Affected neonates rapidly 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 clinical and biochemical manifestations, explaining why aBS is also designed as hyperprostaglandin-E syndrome (HPS) (15). As patients with aBS/HPS fail to respond to loop diuretics such as furosemide, 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. 38-1*). 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 and 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). At least 30 mutations, essentially missense or frameshift, have been described thus far (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). Accordingly, one could speculate that mutations affecting low-capacity/high-affinity isoform might 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 5 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 contribute 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, loss of function in ROMK, as well as in NKCC2, disrupts NaCl reabsorption in the TAL. At least 40 mutations in *KCNJ1* that coseggregate with aBS have been reported, including 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 38-2*). 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, 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 compound heterozygotes for 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 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, 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 1 (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,

Table 38-2

Clinical and biochemical features of Bartter-like syndromes

Feature	aBS (*SLC12A1*) Type I BS	aBS (*KCNJ1*) Type II BS	aBS with SND (*BSND*) Type IV BS	cBS (*CLCNKB*) Type III BS	GS (*SLC12A3*)
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	Rare	Absent
Sensorineural deafness	Absent	Absent	Present	Absent[a]	Absent
Dehydration episodes	Severe	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

[a]SND is present in case of digenic disorder with inactivating mutations of *CLCNKA* and *CLCNKB*

hypokalemia in ROMK-deficient patients is less severe than that observed in NKCC2-patients (29, 31).

Recently, Ji and Lifton reported that heterozygote carriers of inactivating mutations in NKCC2 and ROMK in the general population had significantly lower systolic and diastolic blood pressure, and a significant reduction in the risk of developing hypertension (45). Furthermore, Tobin et al. (46) showed that polymorphisms in *KCNJ1* were associated with blood pressure values in a cohort of 2,037 adults after adjusting for age, sex, and familial correlations. Taken together, these studies suggest that the transporters involved in NaCl handing in the TAL may exert a profound influence on blood pressure regulation (47).

Differential Diagnosis

Antenatal BS should always be suspected in face of polyhydramnios due to fetal polyuria. As discussed above, patients with type II aBS due to ROMK deficiency may show a transient hyperkalemia, which can mimick 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 (48). This association, which probably results from medullary nephrocalcinosis, can mimick incomplete distal renal tubular acidosis (18). Other causes of pseudo-Bartter syndromes will be discussed in the section on cBS.

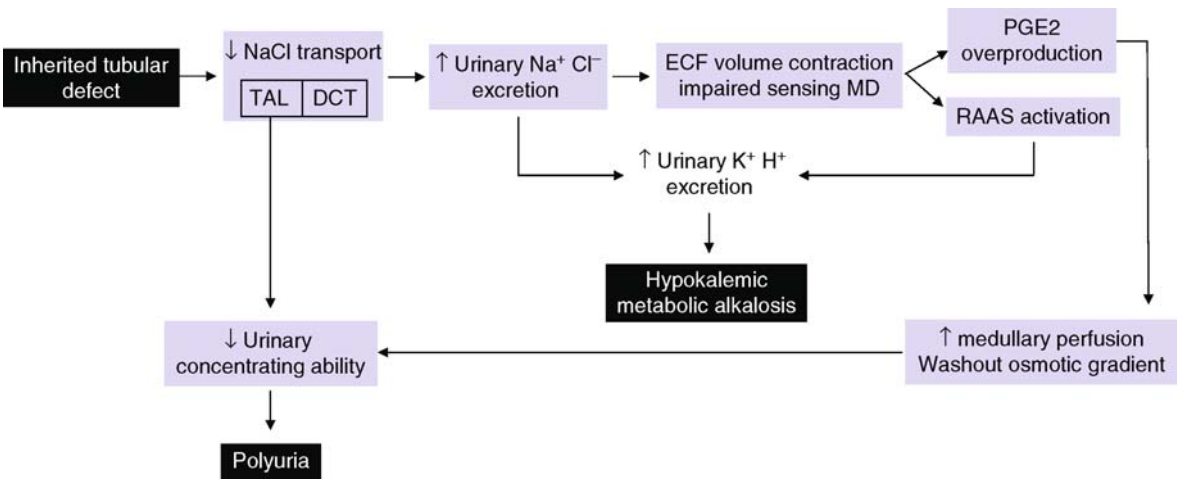
Pathophysiology

Functional investigations of pathogenic NKCC2 mutations in *Xenopus laevis* oocytes revealed a low expression of normally routed but functionally impaired transporters (49). 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 (50). Several 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. (51) identified defective membrane trafficking in 14/20 naturally occurring ROMK mutations, and showed that two early inframe stop mutations could be rescued by aminoglycosides, resulting in full-length ROMK and correct trafficking to the plasma membrane.

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. 38-2*). These changes are already observed prenatally, with polyhydramnios, high Cl^- concentrations and increased aldosterone levels in the amniotic fluid (52, 53). 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 (54). It also appears that cyclooxygenase-2 (COX-2) is induced in the macula densa of children with aBS (55), which could further contribute to hyperreninemia (56). The early neonatal hyperkalemia harbored by some ROMK-deficient patients is explained by the involvement of ROMK in K^+ secretion in the cortical collecting duct, with subsequent

Figure 38-2

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



activation of an alternative pathway for K^+ secretion in the CCD explaining the transient nature of this feature (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 (57). 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 jnction structures (claudin16/claudin19), whereas increased PGE2 synthesis may contribute to increased Mg^{2+} reabsorption in the DCT (58). It has been suggested that a bone resorption process and a PGE2-mediated increase in calcitriol may contribute to hypercalciuria in aBS (35, 59). Recently, Schurman et al. (60) suggested that elevated levels of angiotensin II may stimulate the synthesis 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 (61). Treatment of the NKCC2 KO mice with indomethacin from day 1 allowed 10% mice to survive until adulthood, despite polyuria, hydronephrosis, hypokalemia, and hyperalciuria. Similarly, the ROMK KO mice (62) manifest early death associated with polyuria, polydipsia, and impaired urinary concentrating ability. 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 (63). Recently, Lu et al. used CFTR-deficient mouse models to demonstrate that this 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 (64).

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, 48). 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 to 2.5 mg/kg/day) reduces polyuria, improves hypokalemia, normalizes plasma renin levels, and reduces hypercalciuria (13, 31, 33, 34, 48). However, the potential benefit of indomethacin administration in premature babies and neonates should be weighted against risks of severe gastrointestinal complications, such as ulcers, perforation and necrotizing enterocolitis (13, 33, 65). 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 (66). Similarly, the potential benefit of prenatal treatment with indomethacin (66) 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 (65). Kleta et al. pointed that selective and nonselective cyclooxygenase inhibitors can be used for treatment (66). 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 (56, 67). However, a case of reversible acute renal failure associated with rofecoxib in an 18-month-old girl with aBS has been reported (68).

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, 69) 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, 48). Chaudhuri et al. (39) reported a case of severe aBS in whom pre-emptive nephrectomy followed by a living-related donor renal transplantation resulted in correction of metabolic abnormalities and excellent graft function.

Antenatal Bartter Syndrome with Sensorineural Deafness

Genetics

In 1995, Landau et al. (70) described a subtype of aBS/HPS associated with sensorineural deafness (SND) in 5 affected subjects from an inbred Bedouin kindred. These patients had a particularly severe salt wasting and fluid loss, with poor response to indomethacin and, most often, progressive renal failure (70, 71). By studying the large original kindred, Brennan et al. mapped the disease-causing gene to chromosome 1p31 (72). 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 4 exons. It encodes barttin, a 320 amino-acids 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. 38-1*), and in the stria vascularis surrounding the cochlear duct in the inner ear (8, 73).

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, polyria with hypo- or isosthenuria, and increased urinary PGE2 excretion (2, 74) (*Table 38-2*). All patients are deaf and show a severe growth defect, with delayed motor development (71). They present multiple episodes of fever, vomiting, and bacterial infections. Jeck et al. (71) 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 (74). 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, 74). 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 Mg^{2+} wasting, caused by a defect in both the paracellular (TAL) and transcellular (DCT) pathways of Mg^{2+} 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 (75).

Recent reports have suggested some degree of phenotype variability among patients with *BSND* mutations. Miyamura et al. (76) 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 (77). Kitana et al. (78) 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 (79), possibly accounting for a milder phenotype (74). 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 (71, 74).

Pathophysiology

Functional expression studies revealed that barttin is an essential beta-subunit for the Cl^- channels ClC-Ka and ClC-Kb, by stimulating Cl^- currents and enhancing surface expression of these channels (73). ClC-Ka and ClC-Kb are two members of the CLC gene family that are located on the basolateral membrane of the cells lining the thin ascending limb (ClC-Ka only), TAL and DCT, and the intercalated cells of the collecting duct. ClC-Ka

and ClC-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 (73). The co-expression of barttin with ClC-Ka/b channels is crucial for NaCl reabsorption in the TAL/DCT (*Fig. 38-1*) 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-ClC-Ka/b complex impairs the basolateral recycling of Cl^- in the stria vascularis, decreasing the secretion of K^+ into the endolymph and causing SND (73, 80). The role of barttin as an essential beta-subunit for ClC-Ka and ClC-Kb has been substantiated by two recents reports of patients showing a typical aBS with SND phenotype indistinguishable from barttin-deficient patients, in association with a digenic disease caused by loss-of-function mutations in both *CLCNKB* and *CLCNKA* genes (81, 82).

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 (71, 74). Hypokalemic metabolic alkalosis persists despite high doses of NaCl and KCl supplementation (71). Zaffanello et al. (75) 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 succesful renal transplantation has been reported in a 1-year-old child with type IV BS (39).

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 ClC-Kb, which mediates Cl^- efflux from epithelial cells lining the TAL and DCT (9, 80) (*Fig. 38-1*). There is a high rate of deletions encompassing a part of or the entire *CLCNKB* gene (9, 11, 83). 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 insertions/deletions, frameshift and splice-site mutations have also been reported (http://www.hgmd.cf.ac.uk). A founder mutation (A204T) affecting a highly conserved residue has been reported at the homozygous state in ten patients from nine unrelated, non-consanguinous families in Spain (84). As expected for a recessively transmitted disorder, a significant number of subjects originates from consanguineous kindred (9, 11).

Pathophysiology

As mentioned earlier (see section on barttin), ClC-Kb is a plasma membrane channel that belongs to the CLC family of chloride channels/exchangers (80). ClC-Kb and the closely related ClC-Ka isoform are located on the basolateral membrane of the cells lining the thin ascending limb (ClC-Ka only), TAL and DCT cells, as well as in the intercalated cells of the collecting duct (*Fig. 38-1*). They both require the beta-subunit barttin to facilitate their insertion in the plasma membrane and to generate Cl^- currents (73, 79). Disease-causing missense mutations of ClC-Kb result in significant reductions or the loss of ClC-Kb/barttin currents (73). Thus, inactivating mutations in ClC-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 thus be explained by the wide distribution of ClC-Kb (11, 31). Alternative pathways for Cl^- exit could partially compensate for ClC-Kb inactivation in the kidney (11, 85). Importantly, none of the type III BS patients with ClC-Kb mutations is deaf, because the function of ClC-Kb/barttin channels in the inner ear can be replaced by ClC-Ka/barttin. Only the disruption of the common β subunit barttin (73) or the combined loss of ClC-Ka and ClC-Kb (81, 82) 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 ClC-Kb. Mice lacking ClC-K1 (corresponding to ClC-Ka in humans) show a phenotype of nephrogenic diabetes insipidus, with no modification in the fractional

excetion of Na^+ and Cl^-, and no hypokalemic alkalosis (86). These features are caused by the loss of the Cl^- transport across the thin ascending limb, which is essential for generating a hypertonic interstitium (86). No corresponding human disease linked to loss-of-function mutations of *CLCNKA* has been described. A recent study supported the potential role of *CLCNKA* as a susceptibility gene for salt-sensitivity (87).

Clinical Manifestations

Patients harboring mutations of ClC-Kb present a broad spectrum of clinical features (*Table 38-2*) 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, 84). 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, 84). 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. Most patients with classic BS show failure to thrive and growth retardation (36, 88, 89). 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. 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 persistance of such a concentrating ability suggests that patients lacking ClC-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 (84, 88, 89). 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 (84). About half of the patients lacking ClC-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, 85, 90, 91). Sun et al. (92) 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 (84). No correlations between a particular phenotype and *CLCNKB* genotype have been documented yet (11, 85). 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 (93). More recently, Schurman et al. (89) reported significant phenotype variability in the neonatal period in a series of 5 unrelated African American children with a homozygous deletion of the entire *CLCKNB*.

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 94) showed that this hyporeactivity could be due to various modifications in the angiotensin II-signaling, including downregulation of the alpha subunit of the heterotrimeric Gq protein, decreased intracellular Ca^{2+} and blunted protein kinase C activation, downregulation of the RhoA/Rho kinase pathway, and upregulation of endothelial NO synthase. In turn, these modifications may affect the upregulation of NAD(P)H oxidase and prevent the release of free radicals – offering increased protection against cardiovascular remodeling in these patients (94). Stoff et al. (95) evidenced a defect in platelet aggregation in four subjects with Bartter syndrome, but not in other hypokalmic 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 (96).

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. (97) described a 4-year-old African American child

with a homozygous deletion in *CLCNKB* and mild mesangial proliferative glomerulonephritis consistent with C1q nephropathy. The recent follow-up studies of Bettinelli et al. (88) revealed mild-to-moderate glomerular proteinuria in 6/13 ClC-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 (88). In addition, a few cases of clinical Bartter syndrome with unknown genetic defect presented with focal segmental glomerulosclerosis (FSGS) and renal failure (98, 99). One of the patients described by Bartter developed renal failure, with evidence of advanced nephrosclerosis, interstitial fibrosis, tubular atrophy, and glomerular hyalinization (100). 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, 97, 99). 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, 101). Of note, renal cysts have been identified in patients with classic BS (33, 102), potentially linked to renal K^+ wasting and secondary aldosteronism (103).

Recently, Jeck et al. identified the common T481S variant in *CLCNKB*, which showed significantly increased currents when expressed in oocytes (104) and was associated with essential hypertension in a German cohort (105). The relevance of these findings has been discussed (106), and linkage of the T481S variant to high blood pressure was not confirmed in a Japanese cohort (107).

Differential Diagnosis, Unusual Associations, Pseudo-Bartter Syndromes

The differential diagnosis of cBS includes the surreptitious use of loop diuretics, laxative abuse (108), which are both unusual in children (109), and chronic vomiting (110). Measurement of urinary Cl^- and urine screen for diuretics are usually useful to diagnose these patients (111, 112).

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 (113), or Kearns-Sayre syndrome, a mitochondrial cytopathy caused by large deletions in mitochondrial DNA leading to cytochrome c oxidase deficiency (114), can be associated with biochemical features resembling BS. A case of familial renal dysplasia with hypokalemic alkalosis has been reported (115). Patients with cystic fibrosis are prone to develop episodes of hyponatraemic, hypochloraemic dehydration with metabolic alkalosis (116, 117). As mentioned earlier, the Cl^- channel CFTR, which is mutated in cystic fibrosis, may regulate the function of ROMK in the TAL (64). Gastrointestinal malformations which are associated with Cl^- deficiency (118), or Hirschprung disease (119) can also lead to pseudo-Bartter syndrome. Administration of protaglandins in neonates with a ductus-dependent congenital cardiopathy (120), aminoglycosides (121, 122) or combined chemotherapy (123) can also induce the biochemical features of BS. Bartter syndrome has also been reported in association with autoimmune diseases, for isntance with Sjögren syndrome (124, 125). Güllner et al. (126) 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 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 were 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 (127, 128).

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, 88, 89). 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 gastrointestinal complications (33) or alteration of renal function (38, 88). 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 (89). 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 (129), should be cautious 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 (89).

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, 88, 89). Recently, growth hormone deficiency has been demonstrated in some patients, with a positive effect of recombinant human hormone treatment (88). 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 (98, 101, 130).

Peri-operative management of patients with cBS requires a particular care for volume repletion and correction of electrolyte abnormalities during anesthesia, and the continuation of anti-prostanglandin therapy to prevent the defective platelet aggregation (131, 132).

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 co-workers as a familial disorder in which patients presented with hypokalemic alkalosis and a peculiar susceptibility to carpopedal spasm and tetany due to hypomagnesemia (133). For more than 20 years, GS was assimilated with BS. In 1992 Bettinelli and co-workers 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) (134). 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 (134). The dissociation of renal Ca^{2+} and Mg^{2+} handling in GS, together with the subnormal response of these patients to thiazides (135, 136), 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). The *SLC12A3* gene is located on 16q13 and comprises 26 exons. It encodes the thiazide-sensitive Na^+-Cl^- cotransporter (NCCT), a 1,021 amino-acids integral membrane protein expressed in the apical membrane of cells lining the DCT (*Fig. 38-1*). NCCT belongs to the nine-member family of electroneural cation-chloride coupled cotransporters (*SLC12*) that also includes the Na^+-K^+-$2Cl^-$ and the K^+-Cl^- cotransporters (137). NCCT contains a central hydrophobic region comprising 12 putative transmembrane (TM) domains flanked by a short N-terminal and a long C-terminal hydrophilic intracellular termini (138). A model suggests that the affinity-modifying residues for Cl^- are located within TM 1–7 and for thiazides between TM 8–12 and that both segments are implicated in defining Na^+ affinity of NCCT (139).

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 110 mutations scattered through *SLC12A3* have been identified in GS patients (http://www.hgmd.cf.ac.uk). 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 25% in some series, is found to carry only a single mutation in *SLC12A3*, instead of being compound heterozygous or homozygous (140–142). 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. As discussed above, mutations in *CLCNKB* have been detected in a few patients presenting simultaneous features of cBS and GS (85, 90, 91). The distribution of ClC-Kb in both the TAL and DCT, and potential compensation by other Cl^- transporters, may probably explain these overlaping 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 (143).

Pathophysiology

The functional effects of mutants NCCT were tested using *X. laevis* oocytes (142, 144–146). Functional analyses revealed that some mutant NCCT proteins were synthesized but not properly glycosylated, targeted for degradation, and not delivered to the plasma membrane (144). 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 (145, 146). Some NCCT mutants affect the intrinsic activity of the cotransporter, with normal glycosylation and plasma membrane insertion (142). Finally, splicing mutations of *SLC12A3* result in truncated transcripts that trigger nonsense-mediated decay (NMD), a mRNA surveillance pathway that allows cells to degrade mRNA that contains premature translation stop codons (142). 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 (142).

Schultheis and colleagues generated a mouse model with a null mutation in the *Slc12a3* gene on a mixed background (147). 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 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 (148) and an increased sensitivity to develop hypokalemia when exposed to dietary K^+ reduction (149). More recently, Belge and co-workers 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 (150). They demonstrated that these modifications were due to modifications in intracellular Ca^{2+} signaling and decreased expression of NCCT in the DCT (150).

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 (151). 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 (152). Such hyperpolarization could also stimulate the basolateral Na^+/Ca^{2+} exchanger, further increasing Ca^{2+} reabsorption (153). Studies performed in chronic hydrochlorothiazide-treated mice (154) 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 (148).

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, 155). The recent 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 (156). 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 (154). 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 (147, 157).

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 38-2*). 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 (158). Typical manifestations include muscle weakness, carpopedal spasms, or tetanic episodes triggered by hypomagnesemia (57, 159). Blood pressure is reduced, particularly for patients with severe hypokalemia and hypomagnesemia (160). 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 (161) and sclerochoroidal calcifications (162). 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 (163). Potassium and Mg^{2+} depletion prolong the duration of the action potential in cardiomyocytes, resulting in prolonged QT interval in $\sim$ 50% of the patients, which could lead to an increased risk for ventricular arrythmias (164, 165). Cases of GS patient who presented with long runs of ventricular tachycardia (166) or ventricular fibrillation with favorable outcome after cardioversion and continuous supplementation (167) have been reported. Pregnancies in GS appear to have a favorable outcome, provided continuous K^+ and Mg^{2+} supplementation and monitoring for oligohydramnios (168). A summary of the manifestations associated with GS and their frequency is shown in *Table 38-3*.

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 (134). The criteria for hypocalciuria in infants or children with GS have recently been precised (169). However, there are inter- and intra-individual variations in the extent of hypocalciuria, and hypomagnesemia may be absent in some GS patients (2, 31, 170). In addition, the combination of hypocalciuria and hypomagnesemia is also detected in rare cases of cBS (90). Although the urinary PGE_2 excretion is classically normal in GS, increased values can also be detected in some patients (2).

No specific findings are observed at renal biopsy, apart from occasional hypertrophy of the juxta-glomerular apparatus and markedly reduced expression of NCCT by immunohistochemistry (171). Hanevold et al. (172) reported a case of focal segmental glomerulosclerosis and C1q nephropathy in an African American child with GS who subsequently developed nephrotic range proteinuria.

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

Table 38-3
Clinical manifestations associated with Gitelman syndrome

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

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 (173). 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, 142, 158, 166, 173). Based on the large number of patients harboring *SLC12A3* mutations, the phenotype of GS is highly heterogeneous in terms of age at presentation, nature/severity of biochemical abnormalities, and nature/severity of the clinical manifestations (*Table 38-3*). The phenotype variability has been documented not only between patients carrying different *SLC12A3* mutations, but also for a common underlying mutation (174) and between affected family members (142, 175).

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, Ca^{2+}, or Mg^{2+}) (2, 142, 175, 176). Compensatory mechanisms operating in other nephron segments should also be considered, as evidenced in mice with defective NCCT (148, 150, 154). 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 recent studies of Riveira-Munoz et al. (142) which showed that a specific combination of mutations was preferentially associated with a severe presentation of GS.

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 (160). 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 (177). 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). Recently, Lifton and colleagues screened 3,125 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, ten were biochemically proven loss of function (seven 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 38-1*), and particularly cBS due to mutations in *CLCNKB* (2, 170), 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 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. Recently, Colussi et al. evaluated the response to a simple thiazide test in the diagnosis of GS (170). 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 (170). 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 (178).

Gitelman syndrome-like manifestations including hypokalemic metabolic alkalosis with hypomagnesemia and

hypocalciuria, have been reported as a rare complication of the use of cisplatin (179). Although the mechanism remains uncertain, cisplatin is known to induce focal tubular necrosis lesions in the DCT (180). Autoimmune disorders cause acquired renal tubular disorders, potentially due to autoantibodies against tubular components (181). Typical features of acquired GS have been reported in association with various autoimmune disorders including iritis and arthritis (182), sialoadenitis (125), and Sjögren syndrome (183). Of note, there was no improvement of renal K^+ wasting after corticosteroid treatment in one such case (183).

Treatment

Magnesium and potassium supplementations are the main treatments 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 (159, 184). All types of magnesium salts are effective, but their bioavailability is variable. Magnesium chloride, magnesium lactate and magnesium aspartate show higher bioavailability (159). $MgCl_2$ is recommended since it will also correct the urinary loss of Cl^-. The dose of magnesium must be adjusted individually in 3–4 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 (185). Importantly, Mg^{2+} and K^+ supplementation results in a catchup growth (142, 158). 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 (186). 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. Prostaglandin inhibitors 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 indometacin, but complicated by gastrointestinal haemorrhage (187). Refractory hypokalemia has also been treated with the specific COX-2 inhibitor Rofecoxib (188). Considering the occurrence of prolonged QT interval in up to half GS patients (164, 165), QT-prolonging medications should be used with caution.

Although GS adversely affects the quality of life (160), we lack informations about the long-term outcome of these patients. Renal function and growth appear to be normal, provided lifelong supplementation (189). Progression to renal failure is extremely rare in GS: only two GS patients who developed end-stage renal disease have been reported (190, 191).

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 (192). The human *CASR* gene is located on chromosome 3q21 with a coding region of 3,234 bp and 6 exons (193). The CaSR is a ~120 kD protein forming homodimers through interactions of cysteine residues in the extracellular domain (194). The CaSR is predominantly expressed in the apical membrane of the parathyroid hormone (PTH)-secreting cells in the parathyroids and, 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 (195). 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 (195, 196).

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) (192). Activation of the CaSR mediates different, cell-specific signal transduction pathways (195). 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 (195). Microperfusion studies in rat revealed that elevation of peritubular $[Ca^{2+}_o]$ and $[Mg^{2+}_o]$ markedly reduces the fractional absorption of Ca^{2+}, Mg^{2+}, and Na^+ in the TAL (197). 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. 38-1*). High $[Ca^{2+}_o]$ in the TAL decreases hormone-dependent cAMP accumulation, reflecting a direct inhibition of the CaSR-dependent Galpha-adenylylate cyclase (AC) activity (195). 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 reducing Ca^{2+} and Mg^{2+} transport (198).

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 (199). Loss-of-function CaSR mutations result in familial hypocalciuric hypercalcaemia (FHH) and neonatal severe primary hyperparathyroidism (NSHPT) (200, 201), whereas gain-of-function CaSR mutations result in autosomal dominant hypocalcemia (ADH) (202, 203), which can be associated with a Bartter-like syndrome (204, 205) (*Table 38-4*; *Fig. 38-3*). The prevalence of FHH is up to one in 16,000, ADH one in 70,000, whereas NSHPT is very rare (206).

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 (207). Although patients with FHH are usually asymptomatic, complications such as chondrocalcinosis, acute pancreatitis and gallstones may occur with age (208, 209). A simple diagnostic test is a calcium over creatinine clearance ratio (CCCR) <0.01 (210). 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 (211). 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 (199, 212, 213). Isolated cases of FHH with a de novo mutation in *CASR* have also been reported (214, 215). Many *CASR* mutations cluster in aspartate and glutamate-rich regions of the extracellular domain of the receptor, which may act as cationic binding sites (199). 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 (201, 203). The defective extracellular CaSR likely leads to inappropriate absorption

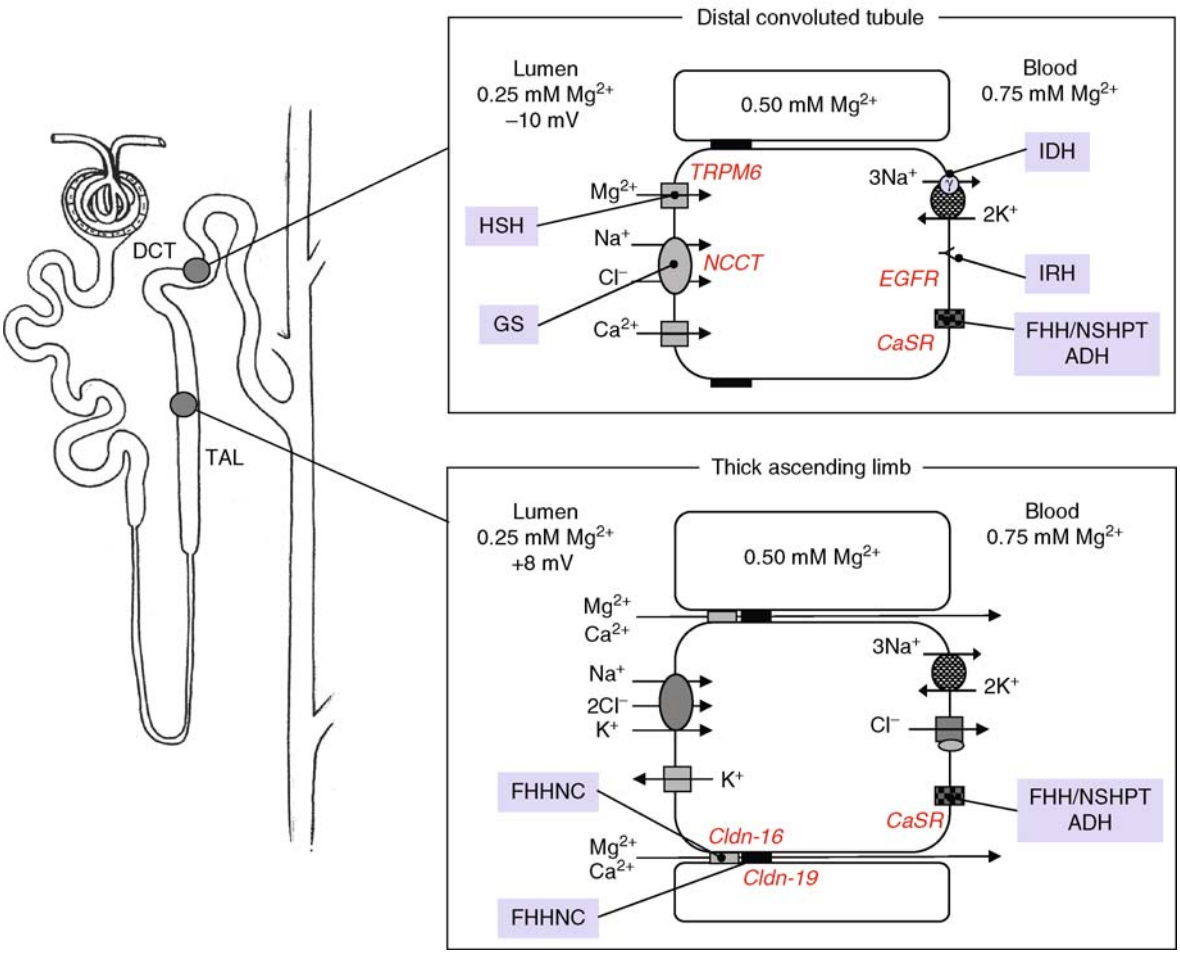
Table 38-4

Inherited disorders of the extracellular Ca^{2+}-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

Figure 38-3

Inherited disorders of magnesium reabsorption in the loop of Henle and distal convoluted tubule. In the thick ascending limb (TAL) of Henle's loop, Mg^{2+} 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), Mg^{2+} is actively reabsorbed via the transcellular pathway involving an apical entry step through a Mg^{2+}-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 gamma-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).



of Ca^{2+} and Mg^{2+} in the TAL (198) and Mg^{2+} transport in the DCT (196). Renal excretion of Ca^{2+} and Mg^{2+} is reduced, which leads to hypercalcemia and sometimes hypermagnesemia (216).

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 (199). 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 (217).

Neonatal severe primary hyperparathyroidism (NSHPT) (OMIM #239200) is a life-threatening, severe hyperparathyroidism characterized by hypercalcaemia, failure to thrive, osteopenia, multiple fractures, and rib cage deformities developing soon after birth (218). NSHPT is usually caused by homozygous *CASR* mutations in children born to consanguineous FBH parents (201, 202). Of note, a marked phenotypic heterogeneity has been observed amongst four members of a kindred harboring the homozygous (Q164X) mutation of *CASR* (219). Patients with sporadic NSHPT have been reported to be associated with de novo heterozygous inactivating mutations of *CASR* (199, 212). 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 knock-out 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 (220). 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 (221). 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 (222). 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 (221).

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 remains challenging and requires specific measures. The acute management of hypercalcemia classically relies on saline perfusion and careful use of loop diuretics. Pamidronate, a biphosphonate 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 (219). Radical subtotal parathyroidectomy is often the treatment of choice in NSHPT (223). 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 (224). 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 (225). 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 (215).

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) (202, 203). 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 (199, 203). 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 (57).

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 (199). 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+} (203). 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 (195). Hormone-stimulated Mg^{2+} reabsorption is also inhibited in the DCT, which probably contributes to the renal magnesium loss (155).

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 (199, 203). Therefore, the treatment of hypocalcemia in ADH with vitamin D and calcium supplementation should be restricted to clearly symptomatic patients (57). 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 (226).

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., development of a salt-losing tubulopathy associated with urinary concentrating defect and hypokalemic metabolic alkalosis (204, 205). 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 (204, 205). 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. 38-1*). 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 (227).

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 (228, 229). 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 (230). 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 lumen-positive transepithelial voltage (*Fig. 38-3*). Only 5–10% of the filtered Mg^{2+} is reabsorbed in the DCT. However, in this part of the nephron the fine adjustment of renal excretion is accomplished. In the DCT, Mg^{2+} transport is an active transcellular process (*Fig. 38-3*). Physiologic studies indicate that apical entry into DCT cells is mediated by the specific and regulated Mg^{2+} channel TRPM6. The mechanism of basolateral transport into the interstitium is unknown. Here, Mg^{2+} has to be extruded against an unfavorable electrochemical gradient. Most physiologic studies favor a Na^{+}-dependent exchange mechanism (231). Mg^{2+} entry into DCT cells appears to be the rate-limiting step and the site of regulation. For details of Mg^{2+} transport in the distal tubule see Dai et al. (155). In the collecting duct, there is no significant Mg^{2+} uptake. Finally, 3–5% of the filtered Mg^{2+} 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, cisplatine, calcineurin inhibitors). During infancy and childhood, a substantial proportion of patients receiving medical attention for signs of hypomagnesemia are affected by inherited renal disorders associated with Mg^{2+} wasting. In these disorders hypomagnesemia may either 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 Mg^{2+} transport, and helped to characterize different clinical subtypes of hereditary Mg^{2+}-wasting (Table 38-5). A careful clinical and biochemical assessment allows to distinguish the different disease entities in most cases, even when there is a considerable overlap in the phenotypic characteristics (Table 38-6).

Gitelman Syndrome

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

Isolated Dominant Hypomagnesemia

Isolated dominant hypomagnesemia (IDH, OMIM #154020) results from a mutation in the *FXYD2* gene on chromosome 11q23 which encodes a γ-subunit of the Na^+-K^+-ATPase (232). Only two IDH families have been described so far (233, 234). In both families, 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. Serum Mg^{2+} measurements performed in members of both families revealed low serum Mg^{2+} levels (around 0.5 mmol/L) in numerous apparently healthy individuals. A ^{28}Mg-retention study in one of the patients indicated a primary renal defect (233). 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. In the two families, hypomagnesemia was inherited as an autosomal dominant trait. A genome-wide linkage study could map IDH to chromosome 11q23 (235). Detailed haplotype analysis demonstrated a common

Table 38-5
Inherited disorders of renal magnesium handling

Disorder	OMIM #	Inheritance	Gene locus	Gene	Protein
Gitelman syndrome	263800	AR	16q13	*SLC12A3*	NCCT, Na^+-Cl^- cotransporter
Isolated dominant hypomagnesemia	154020	AD	11q23	*FXYD2*	γ-subunit of the Na^+-K^+-ATPase
Isolated recessive hypomagnesemia	611718	AR	4q25	*EGF*	Pro-EGF, epidermal growth factor
Autosomal dominant hypocalcemia, Autosomal dominant hypoparathyroidism	146200	AD	3q21	*CASR*	CaSR, Ca^{2+}/Mg^{2+}sensing receptor
Familial hypocalciuric hypercalcemia, Familial benign hypercalcemia	145980	AD	3q21	*CASR*	CaSR, Ca^{2+}/Mg^{2+} sensing receptor
Neonatal severe primary hyperparathyroidism	239200	AR	3q21	*CASR*	CaSR, Ca^{2+}/Mg^{2+} sensing receptor
Familial hypomagnesemia with hypercalciuria/ nephrocalcinosis	248250	AR	3q28	*CLDN16*	Claudin-16 (paracellin-1), tight junction protein
Familial hypomagnesemia with hypercalciuria/ nephrocalcinosis and severe ocular involvement	248190	AR	1p34	*CLDN19*	Claudin-19, tight junction protein
Hypomagnesemia with secondary hypocalcemia	602014	AR	9q22	*TRPM6*	TRPM6, Mg^{2+} channel
Hypomagnesemia/metabolic syndrome	500005	maternal	mtDNA	*MTTI*	Mitochondrial tRNA (Isoleucin)

Table 38-6

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+}	Nephro-calcinosis	Renal stones
Gitelman syndrome	Adolescence	↓	N	↓	↑	↑	↓	No	no
Isolated dominant hypomagnesemia	Childhood	↓	N	N	N	↑	↓	no	no
Isolated recessive hypomagnesemia	Childhood	↓	N	N	N	↑	N	no	no
Autosomal dominant hypocalcemia, Autosomal dominant hypoparathyroidism	Infancy	↓	↓	N	N or ↓	↑	↑ – ??	yes[a]	yes[a]
Familial hypocalciuric hypercalcemia, Familial benign hypercalcemia	Often asymptomatic	N to ↑	↑	N	N	↓	↓	no	?
Neonatal severe primary hyperparathyroidism	Infancy	N to ↑	↑↑↑	N	N	↓	↓	no	?
Familial hypomagnesemia with hypercalciuria/nephrocalcinosis	Childhood	↓	N	N	N or ↓	↑↑	↑↑	yes	yes
Hypomagnesemia with secondary hypocalcemia	Infancy	↓↓↓	↓	N	N	↑	N	no	no

[a]frequent complication during therapy with Ca^{2+} and vitamin D

haplotype segregating in the two families suggesting a common ancestor. Subsequent mutation analysis of the *FXYD2* gene demonstrated the identical mutation G41R in all affected individuals of both family branches (232).

The protein encoded by *FXYD2* is a member of a small single transmembrane protein family which share the common amino acid motif F-X-Y-D. FXYD proteins modulate the function of the ubiquitous Na^+-K^+-ATPase, a dimeric enzyme invariably consisting of one α- and one β-subunit. FXYD proteins constitute a third or γ-subunit that represents a tissue-specific regulator of the Na^+-K^+-ATPase. Two members of this family, FXYD2 and FXYD4, are highly expressed along the nephron displaying an alternating expression pattern (236). 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 (237). The FXYD2 γ-subunit increases the apparent affinity of Na^+-K^+-ATPase for ATP while decreasing its Na^+ affinity (237). 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 (234). Urinary Mg^{2+} wasting together with the expression pattern of the *FXYD2* gene indicate defective transcellular Mg^{2+} reabsorption in the DCT in IDH patients. 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 (232). Alternatively, an increase in intracellular Na^+ could impair basolateral Mg^{2+} transport which is presumably achieved by a Na^+-coupled exchange mechanism. Another explanation is that the γ-subunit is not only involved in Na^+-K^+-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 Mg^{2+}-ATPase and a Na^+-coupled exchanger might exist. Further studies are needed to clarify this issue.

An interesting feature of IDH is the finding of hypocalciuria which is primarily observed in GS. Unfortunately, only one large family with IDH has been described and an animal model for IDH is still lacking. Mice lacking the γ-subunit (*Fxyd2*) do not demonstrate significant abnormalities in Mg^{2+} conservation or balance (238). One could speculate that, like in GS, a defect in Na^+-K^+-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, 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 (154).

Isolated Recessive Hypomagnesemia

Geven and colleagues reported a form of isolated recessive hypomagnesemia (IRH, OMIM #611718) in a consanguineous family (239). 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 (239). Both patients exhibited renal Mg^{2+} excretion of 3–6 mmol per day despite hypomagnesemia confirming renal Mg^{2+} wasting. In contrast to IDH, 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 (240). The EGF protein is expressed in the DCT, and its binding to the receptor EGFR is essential for the function of the TRPM6 channel. 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 (240). 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.

Familial Hypomagnesemia with Hypercalciuria and Nephrocalcinosis

Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC, OMIM #248250) is an autosomal recessive tubular disorder. Since its first description by Michelis et al. in 1972 (241), numerous kindreds have been reported, allowing a comprehensive characterization of the clinical spectrum of this disorder and discrimination from other Mg^{2+} losing tubular diseases (242–246). As a consequence of excessive renal Mg^{2+} and Ca^{2+} wasting, patients develop the characteristic triad of hypomagnesemia, hypercalciuria and nephrocalcinosis that gave the disease its name. Most FHHNC patients present during early childhood with recurrent urinary tract infections, polyuria/polydispsia, nephrolithiasis, and/or failure to thrive. Clinical signs of severe hypomagnesemia are less common. Extrarenal manifestations, especially ocular involvement (including severe myopia, nystagmus, or chorioretinitis) have been reported (244–246). Additional laboratory findings include elevated serum PTH levels before the onset of chronic renal failure, incomplete distal tubular acidosis, hypocitraturia, and hyperuricemia, which are present in most patients (247). The clinical course of FHHNC patients is often complicated by the development of renal failure during the first two decades of life, and about one third of patients develop ESRD during adolescence. Due to a reduction in filtered Mg^{2+} that limits urinary Mg^{2+} excretion, hypomagnesemia may completely disappear with the decline of GFR.

Beside continuous Mg^{2+} supplementation, therapy aims to reduce Ca^{2+} excretion by using thiazides to prevent the progression of nephrocalcinosis and stone formation. The degree of renal calcification has been correlated with progression of chronic renal failure (245). In a short term study, thiazides have been demonstrated to effectively reduce urinary calcium excretion in FHHNC patients (248). However, these therapeutic strategies have not been shown yet to significantly influence the progression of renal failure. Supportive therapy is important for protecting kidney function and should include provision of sufficient fluids and whenever possible, the prevention and/or effective therapy of urinary tract infections. Renal transplantation does not result in recurrence of the disease because the primary defect resides in the kidney.

By positional cloning, Simon et al. identified a new gene on 3q27 (*CLDN16*, formerly *PCLN1*), which is mutated in patients with FHHNC (249). *CLDN16* codes for claudin-16, a member of the claudin family. More than 20 claudins identified so far comprise a family of ~22 kD 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 claudins confers the characteristic properties of

different epithelia for 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. 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 containing the putative ion selectivity filter. Within this domain, patients originating from Germany and Eastern European countries exhibit a common mutation (L151F) due to a founder effect (247). As this mutation is present in approximately 50% of mutant alleles, molecular diagnosis is greatly facilitated in patients originating from these countries.

Family analysis revealed that carriers of heterozygous *CLDN16* mutations may also present with clinical symptoms. Two independent studies describe a high incidence of hypercalciuria, nephrolithiasis and/or nephrocalcinosis in first degree relatives of FHHNC patients (245, 247). A subsequent study also reported a tendency towards mild hypomagnesemia in family members with heterozygous *CLDN16* mutations (250). 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 (251). Interestingly, the hypercalciuria disappeared during follow-up and urinary Ca^{2+} levels reached normal values beyond puberty. Transient transfection of Madine Darby canine kidney (MDCK) cells with the *CLDN16* (T303R) mutant revealed a mistargeting into lysosomes whereas wildtype claudin-16 was correctly localized to tight junctions. It still remains to be determined why this type of misrouting is associated with transient isolated hypercalciuria without increased Mg^{2+} excretion.

The exact physiological role of claudin-16 is still not fully understood. From the FHHNC disease phenotype, it was concluded that claudin-16 might regulate the paracellular transport of Mg^{2+} and Ca^{2+} ions by contributing to a selective paracellular conductance by building a pore permitting paracellular fluxes of Mg^{2+} and Ca^{2+} down their electrochemical gradients (249, 252). However, recent functional studies in porcine renal tubule epithelial kidney cells (LLC-PK1) cells could show that the expression of claudin-16 selectively and significantly increased the permeability of Na^{+} with a far less-pronounced change of Mg^{2+} flux. From these observations, it was hypothesized that in the TAL claudin-16 probably contributes to the generation of the lumen-positive potential (allowing the passive reabsorption of divalent cations) rather than to the formation of a paracellular channel selective for Ca^{2+} and Mg^{2+} (253).

As mentioned above, many FHHNC patients develop chronic renal failure associated with progressive tubulointerstitial nephritis. The pathophysiology of this phenomenon, which is not usually observed in other tubular disorders is unclear. Traditionally, renal failure in FHHNC has been attributed to the concomitant hypercalciuria and nephrocalcinosis, but a true correlation has not been established. Therefore, it has been speculated that claudin-16 is not only involved in paracellular electrolyte reabsorption but also in tubular cell proliferation and differentiation (254). This hypothesis is supported by the bovine *CLDN16* knockout phenotype, which exhibit early onset renal failure due to interstitial nephritis with diffuse zonal fibrosis (255, 256). Tubular epithelial cells were reported as "immature" with loss of polarization and attachment to the basement membrane. A close association between fibrosis and abnormal tubules was noted, and the term "renal tubular dysplasia" was used to emphasize that the lesions develop first in the epithelial cells of the renal tubules (257). These cattle have large homozygous deletions whereas human FHHNC mutations are mainly missense mutations affecting the extracellular loops of claudin-16. From these observations it appears that the site and extent of the mutation determines the phenotypic manifestation ranging from isolated alterations in channel conductance to an alteration in cell proliferation and differentiation. This hypothesis is consistent with the results of a large retrospective study in which the clinical course of FHNNC in more than 70 patients was compared to the functional analysis of the underlying *CLDN16* mutations (258). This study could demonstrate that patients carrying complete loss of function mutations on both alleles are younger at disease onset and have a much more rapid decline of GFR than those patients with at least one mutant allele which displays residual function (258).

FHHNC is genetically heterogenous, since mutations in another tight junction gene encoding claudin-19 have also been demonstrated to cause this disease (259). The identification of *CLDN19* mutations could explain the variable ocular phenotype, because *CLDN19* defects seem to be invariably associated with severe ocular abnormalities (including severe myopia, nystagmus, or macular coloboma) (244–246). 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 (247).

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 (259). 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 (260). This is most likely due to anion-blocking properties of claudin-19 preventing back diffusion of Cl^- anions to the tubular lumen.

Hypomagnesemia with Secondary Hypocalcemia

Hypomagnesemia with secondary hypocalcemia (HSH, OMIM #602014) is a rare autosomal recessive disorder first described in 1968 (261). It manifests in early infancy with generalized seizures or other symptoms of increased neuromuscular excitability. Delayed diagnosis or noncompliance with treatment can be fatal or result in permanent neurological damage. 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 (262). 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+} (263). Transport studies in HSH patients indicated a primary defect in intestinal Mg^{2+} absorption (264). However, in some patients an additional renal leak for Mg^{2+} was suspected (265).

A gene locus (*HOMG1*) for HSH had been mapped to chromosome 9q22 in 1997 (266). Later, two independent groups identified *TRPM6* at this locus and reported loss of function mutations, mainly truncating mutations, as the underlying cause of HSH (156, 267). Subsequently, additional HSH mutations in *TRPM6* have been identified (268, 269). *TRPM6* encodes a member of the transient receptor potential (TRP) family of cation channels. The TRPM6 protein is homologous to TRPM7, a Ca^{2+} and Mg^{2+} permeable ion channel regulated by Mg-ATP (270). 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 (271) confirming that renal Mg^{2+} wasting could play a role in the pathogenesis of HSH (272). This was also supported by intravenous Mg^{2+} loading tests in HSH patients, which disclosed a considerable renal Mg^{2+} leak (267).

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 2,022 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 (156). 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 (271). 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+} (271). 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 (270). Chubanov et al. reported that TRPM6 is only present at the cell surface when associating with TRPM7 (273). 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 coexpression of TRPM6 results in a significant amplification of TRPM7-induced currents (273). Schmitz et al. (274) 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 (274).

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 (240). 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 (275). Similarly, 17-beta-Estradiol induces an upregulation of TRPM6, as shown in ovariectomized rats (275). 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 (276, 277).

Mitochondrial Hypomagnesemia

A mutation in the mitochondrial-coded isoleucine tRNA gene, $tRNA^{Ile}$ or MTTI, related to hypomagnesemia has been discovered in a large Caucasian kindred (278). 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 mitochondrial mutation observed in the affected family involves the $tRNA^{Ile}$ gene *MTTI*. The observed nucleotide exchange 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 (278). 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 (279). 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 continous infusion of 100–200 mg Mg^{2+} sulfate (0.4–0.8 mmol Mg^{2+}) per kilogram body weight per day (280). 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 body weight given three to four times a day has been recommended to correct hypomagnesemia (281). 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 (282). 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 (283, 284). Their beneficial effect on renal Mg^{2+} excretion, serum Mg^{2+} levels and clinical symptoms is well documented in hereditary Mg^{2+}-wasting diseases (186, 285).

Low Renin Hypertension with Hypokaliemia

Glucocorticoid-Remediable Aldosteronism

Glucocorticoid-remediable aldosteronism (GRA, OMIM #103900), also called dexamethasone-suppressible hyperaldosteronism (DSH) or familial hyperaldosteronism type I (FH-I) is a rare but fascinating disease. It was first individualized by Sutherland and coworkers in 1966 (286) and since then has been reported in less than 100 unrelated cases (287, 288).

Clinical Features

Individuals with GRA 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 (289). 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. Indeed, analysis of affected kindreds has shown a high prevalence of hemorrhagic stroke and ruptured intracranial aneurysms, usually before the age of 40 years (290). The biological profile of affected subjects suggests a primary aldosteronism but GRA patients can be normokaliemic. A specific feature of the disease is the aldosterone hyperresponsiveness to maneuvers stimulating or inhibiting the cortisol hypopituitary axis (291). 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 (292, 293). The second specific feature is the abundant urinary excretion of 18-hydroxycortisol and 18-oxocortisol (294). However, dosages of these steroids require sophisticated methods and antibodies and are not performed routinely.

Genetics

In 1992, Lifton and colleagues (295) showed that GRA 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 11β-hydroxylase (CYP11B1) promoter with the coding sequence of aldosterone synthase. In all families reported so far, the chimaeric gene derives from unequal homologous recombination between intron 1 and intron 4 of the *CYP11B1* and *CYP11B2* genes, respectively. This recombination takes always place upstream of exon 5, since this exon contains two residues that differ between the two homologous enzymes (296) and that are critical to confer the aldosterone synthase specificity. Thus, it encodes a protein that can hydroxylate 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, which expression is under ACTH control and can be down-regulated by exogenous glucocorticoid administration (297). 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-blotting or on a long-range PCR looking for the existence of an hybrid gene containing the 5′ part of the CYP11B1 gene and the 3′ part of the CYP11B2 gene (298). 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 an hyperaldosteronism and a positive familial history of early hypertension and/or stroke, should encourage to perform this genetic test.

Therapy

Taking into account this mechanism, the treatment of GRA is based on the administration of dexamethasone at low doses (0.5 mg/day), which only partially suppresses ACTH but lowers the possible side effects from long-term exogenous glucocorticoid treatment. A complementary treatment based on amiloride or spironolactone at low dose as well other classical antihypertensive agents is often required (299).

Other Forms of Familial Aldosteronism

GRA is probably very rare. In our experience, we only detected six unrelated cases amongst 700 patients with primary aldosteronism (X. Jeunemaitre, unpublished data).

Gordon and Stowasser described another form of familial primary aldosteronism, called type II (FH-II). It was initially detected in few families with about one-third

of the affected patients presenting an aldosterone-producing tumor (300). The clinical and biological characteristics of these patients do not differ from those with usual primary aldosteronism, except that the trait seems inherited according to an autosomal dominant transmission with partial penetrance (301). The screening of the entire genome in a large family with FH-II showed linkage with chromosome 7p22 (302). In addition to two Australian kindreds, two other Italian families with FH-II were found to be linked to this locus (303), but no causal gene has been identified yet.

Very recently, a new familial form of aldosteronism has been reported in one single family (304) that could be due to a new gene regulating adrenal steroid biosynthesis. A father and his two daughters had very early (by the age of 7) and severe hypertension with hyperaldosteronism. Very high levels of 18-oxocortisol and 18-hydroxycortisol were not influenced by dexamethasone and GRA was excluded.

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 (305) (OMIM #177200). Although blood pressure and hypokaliemia were not influenced by spironolactone treatment, 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 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 (306), making the epithelial amiloride-sensitive Na^+ channel (ENaC) located in the cortical CD an attractive candidate gene for Liddle syndrome.

Genetics

Analyzing the original Liddle's pedigree, Shimkets et al. (307) 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 for 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 (308). Measurement of transnasal potential difference, as an alternative to transepithelial transport in the kidney, showed the presence of an increased amiloride-sensitive conductance in the three affected boys but not in their unaffected sister (309). 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's syndrome (310). No mutation of αENaC has been associated with Liddle syndrome, yet.

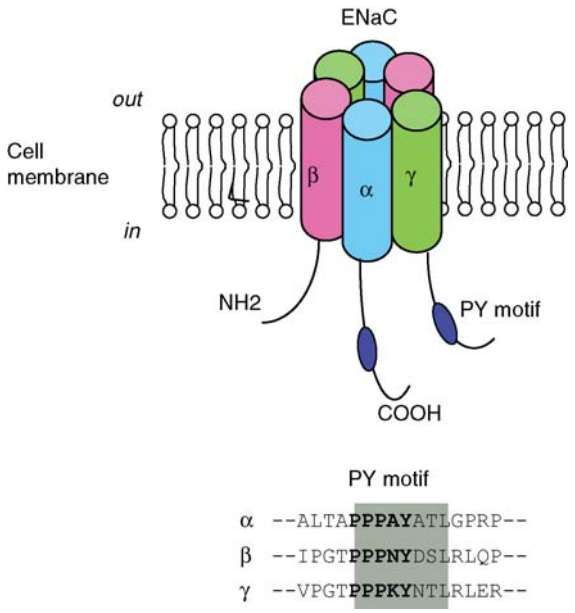
Pathophysiology

ENaC is constituted of at least three homologous subunits, α, β and γ which act together to confer its low Na^+ conductance, and its high selectivity for Na^+ and amiloride (311). The stochiometry of the channel can be deduced from the crystal structure of a chicken acid-sensing ion channel which belongs to the same family (312). It is an heterotrimeric protein composed of one α, β and γ subunit (*Fig. 38-4*). Each subunit is composed of short amino and carboxy termini, two transmembrane helices, and a multidomain extracellular region. ENaC is located in the apical membrane of epithelial cells and plays a major role in the reabsorption of Na^+ ions, especially in kidney, colon and lung. In the kidney, it is mainly expressed in the distal part of the DCT and in the cortical CD. It is finely tuned by changes in salt regimen, thus allowing appropriate changes in Na^+ reabsorption (313).

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 (314, 315). Normally, 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 part of the newly synthetized subunits (316). Thus, cell surface expression of ENaC is in part controlled via ubiquitylation which it itself regulated by aldosterone-induced proteins and glucocorticoid induced

Figure 38-4

Membrane topology of the epithelial Na^+ channel ENaC. The proposed model is a heterotrimeric structure by one α subunit, one β subunit, and one γ subunit. Each subunit is formed by two transmembrane domains, a large extracellular loop and cytoplasmic amino – and carboxy termini. The C-terminus contains a PY motif with at least three proline and a tyrosine residues (PPPXYXXL), highly conserved between the α, β, and γ subunits and between species. This motif is essential for the interaction with intracytoplasmic proteins (among them Nedd4–2) and the regulation of the number of channels at the plasma membrane.



kinase 1 (317). Both truncation or punctual mutation of the C-terminal PY motif increased surface expression of the mutant proteins and thus increased the number of Na^+ channels in the apical membrane (318), favoring renal Na^+ absorption and hypertension. This results in expanded plasma volume which in turns 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.

It has been suggested that polymorphisms at each *SCNN1A*, *SCNN1B* and *SCNN1G* genes could be related to essential hypertension (319). However, the in vitro demonstration of their functionality has proven to be difficult (320). A special emphasis has been put on the Thr574Met polymorphism at the βENaC which frequency is higher (around 8%) in the African populations. A higher frequency of the 574Met allele has been suspected in black hypertensives living in London compared to normotensives (321), this allele being associated with lower plasma renin values, and possibly with an enhanced sensitivity to amiloride (322). Other polymorphisms at the γENaC have also been associated to essential hypertension (323, 324).

Diagnosis

Recognition of Liddle syndrome is important because it is potentially cured by the administration of amiloride. It is a form of 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. As a consequence of the high penetrance of this genetic defect and its autosomal dominant transmission, one can usually find the presence of hypertensive individuals in successive generations. As well, affected individuals are diagnosed at a relatively young age, most often between the age of 10 and 30 years (325). These features are clearly different from the more severe and recessively transmitted apparent mineralocorticoid excess (see below). It resembles to the very rare (only one family described) form of hypertension secondary to activated mineralocorticoid receptor, both forms being not sensitive to spironolactone (see also below). In any case 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 K^+-sparing diuretic (326), 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 (327). 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 (308). In our experience, chronic therapy with amiloride alone is

sufficient to control blood pressure. Spironolactone has no effect which is expected since renin and alosterone are completely suppressed.

Early-Onset Hypertension Secondary to a Gain of Function Mutation in the Mineralocorticoid Receptor

One unique case of a new monogenic form of hypertension, that could be called pseudoaldosteronism type II, has been reported by Geller and colleagues (328). It is characterized by an early-onset and severe form of hypertension, associated with low plasma levels of renin and aldosterone, with severe exacerbation in pregnancy (OMIM #605115). It is caused by an activating missense mutation (Ser810Leu) of the *MR* (or *NR3C2*) gene that encodes the mineralocorticoid receptor. 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. Thus, spironolactone acts an angonist on the mutated receptor instead of an antagonist and could be detrimental. In the family described by Geller and coworkers, all women bearing the mutation had severe pregnancy-induced hypertension with hypoaldosteronism which was caused by the massive increased production of progesterone during pregnancy (328). These original findings open the possibility of discovering other cases of similar type of mutations in pregnancy-induced hypertension. However, several groups including ours (unpublished) failed to find such activating mutation in large series of pre-eclamptic women.

Apparent Mineralocorticoid Excess

The syndrome of apparent mineralocorticoid excess (AME) is a rare autosomal recessive form of hypertension (OMIM #218030). It was first described by New (329) and Ulick (330) in two subjects: one was a 3-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, establishing a new syndrome. AME is usually diagnosed within the first years of life and is characterized by polyuria and polydipsia, a failure to thrive, a severe hypertension associated with hyporeninism and hypoaldosteronism, a profound hypokalemia with alkalosis and most often nephrocalcinosis (331). The syndrome is rare, since less than 100 cases have been reported in the last 30 years. 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 # 207765), have also been reported, with less caricatural hypertension and only mild abnormalities of cortisol metabolism. It was first described by Ulick (332) and later in an extensive consanguineous Sardinian pedigree in whom Li et al. found a novel homozygous mutation in the *HSD11B2* gene (333). 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-beta-hydroxysteroid dehydrogenase is a microsomal enzyme complex responsible for the interconversion of cortisol and cortisone. 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-beta-dehydrogenase activity and thus only catalyzes the cortisol to cortisone reaction (334). Edwards and colleagues (335) showed that the HSD11B2 isoform is highly concentrated in aldosterone-responsive tissues – particularly in the distal nephron -, and actually protects the mineralocorticoid receptor from a stimulation by the cortisol which 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 (336).

The group of White first showed that AME is due to mutations in the *HSD11B2* gene that encodes the HSD11B2 isoform (337). They screened the five exons of *HSD11B2* in nine affected individuals and found missense and frameshift mutations which markedly affected enzymatic activity in vitro and were associated with increased urinary (THF/THE) ratios, the more severe mutations resulting in the higher precursor/product ratios. Subsequently, other loss-of-function mutations have been found in affected patients being either homozygous for the mutation – especially in consanguineous families – or

composite heterozygous (331, 338–340). HSD11B2-null mice provide a good model for the pathology (341). They show signs of hypertension, hypotonic polyuria, hypokalemia and hypochloremia, a phenotype directly comparable to AME patients. They confirm the crucial importance of the HSD11B2 isoform in metabolizing cortisol to cortisone in the renal tubule, thus protecting the mineralocorticoid receptor from the influence of cortisol.

Differential Diagnosis

Consumption of natural licorice can mimick an AME in adults but is exceptionally observed in children. Indeed, it requires either a sustained and chronic or a high acute exposure to cause this adverse effect. The mechanism behind this effect is the fact that licorice contains glycyrrhetinic acid and glycyrrhizic acid, the latter being a potent inhibitor of HSD11B2 (342). These molecules are mostly excreted in the bile together with their metabolites, with very little excretion in urine, explaining the need of an important and chronic consumption to lead to a mineralocorticoid form of hypertension. Carbenoxolone, an antiulcer drug, is also a competitive inhibitor of HSD11B2, and cause sodium retention and hypertension (343).

Treatment

Two main strategies can be used to treat AME. The first is the blockade of the mineralocorticoid receptor by spironolactone, thus acting as a competitive antagonist of the endogeneous cortisol. Daily doses of spironolactone between 2 and 10 mg/kg are usually sufficient to correct hypertension and increase natriuresis and renin levels (331). The addition of thiazides can help to normalize blood pressure and lower hypercalciuria and nephrocalcinosis. The second, complementary strategy consists in administering exogenous corticoids to block ACTH and suppress the endogeneous secretion of cortisol. This strategy has shown its efficiency on blood pressure, renin and aldosterone but has little effect on urinary concentrations of the metabolites of cortisol, cortisone and corticosterone (344). Interestingly, a 31-years-old women with AME was cured by renal transplantation while receiving also dexamethasone. The curative effect of renal transplantation on the cortisol/cortisone ratio confirmed that AME as a renal disorder and that the transplanted kidney had functional HSD11B2 activity (345). In addition to these two strategies, the use of non-specific antihypertensive agents such as calcium antagonists is often required in AME, due to the severity of hypertension (346).

Pseudohypoaldosteronism Type II, Gordon Syndrome

Pseudohypoaldosteronism type II (PHA2) (OMIM #145260), also known as Gordon syndrome, or familial hyperkalemic hypertension, is an autosomal dominant form of volume-dependent hypertension characterized by hyperkaliemia and hyperchloremic acidosis despite normal renal function (347). Since the first description of the disease by Paver and Pauline in 1964 (348), about 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 (347). The original case was a 15-year-old boy with severe hypertension (180/120 mmHg) and very high potassium levels (7.0–8.2 mmol/L). Detailed analyses showed that the kidney was probably involved but that the renal tubule reacted normally to an acid load and to carbonic anhydrase inhibitor. Sensitivity to thiazide diuretics was reported a few years later in unrelated affected subjects (349). A high variability in the age at diagnosis, which may range from the first few weeks of life until late in adulthood, has been reported in sporadic and familial cases. Usually, the biochemical abnormalities precede the increase in blood pressure which seems to depend primarily on age in affected individuals (350). This phenotypic variability, associated with sensitivity to thiazides — which are widely used in hypertension — may have led to an underestimation of PHA2 frequency.

The low renin levels in PHA2 are thought to be the consequence of volume expansion whereas plasma aldosterone levels are variable depending on the opposite influences of low renin and high potassium levels. At least two arguments suggest a primary renal tubular defect along the DCT: affected patients are highly sensitive to thiazide diuretics (349) and the clinical and biochemical features of PHA2 are the mirror image of Gitelman syndrome in which inactivating mutations in NCCT have been demonstrated (see above). However, the pathophysiology of PHA2I is certainly more complex as it has been associated in some cases with defective proximal reabsorption, impaired chloride reabsorption, and altered sensitivity to mineralocorticoids (351).

Genetics

The first genome scan was reported by Lifton's group (352). Using eight affected but limited families, they identified two loci on chromosome 1 (1q31-q42) and chromosome 17 (17q21-q22), respectively named PHA2A and PHA2B

(Fig. 38-5). The selection and genetic analysis of a large French pedigree led us to identify a third chromosomal region on chromosome 12p13, called PHA2C (353). Exclusion of linkage for these 3 chromosomal regions in two additional affected pedigrees suggests further genetic heterogeneity (354). Thus, it is expected that molecular genetics of this syndrome will lead to a variety of molecular defects, revealing the role of either several new components of the same pathway, or direct or indirect partners of the sodium-chloride cotransporter.

The two genes, *WNK1* and *WNK4*, corresponding to the PHA2B and PHA2C loci were identified in 2001 (355) (Fig. 38-6). Both genes are members of a particular family of serine-threonine kinases, called With No Lysine (WNK) kinase (356). Disease-causing mutations in the *WNK1* gene are large deletions in the first intron which lead to increased gene expression of the kidney isoform. Mutations in the *WNK4* gene are missense mutations clustering in a highly conserved domain among this type of kinases. Altogether, these findings implicate these kinases in a previously unrecognized signaling pathway likely to be involved in the control of Na^+ reabsorption in the distal nephron and blood pressure regulation (357).

Pathophysiology

The mechanism of PHA2 has been debated for many years. The two main suggested mechanisms – excessive Na^+ reabsorption via the thiazide-sensitive cotransporter NCCT in the DCT, or a "chloride shunt" that would favor Na^+ reabsorption via ENaC (351) – have been revisited according to the recent identification of *WNK1* and *WNK4* genes as causing the disease (358).

WNK4 seems to be a major player in regulating Na^+, K^+, and Cl^- reabsorption in the distal nephron by regulating regulation of a number of renal ion transporters and channels. In vitro experiments showed that it inhibits NCC activity in *Xenopus* oocytes by decreasing its surface expression and that mutated WNK4 has lost this ability to regulate NCCT (359). It could also inhibit the activity of the renal apical K^+ channel ROMK, the basolateral isoform of the Na^+-K^+-$2Cl^-$ cotransporter (NKCC1), the apical $Cl^-/HCO3^-$ exchanger CFEX and TRPV4 (360), again by decreasing their surface expression. Finally, WNK4 was shown to stimulate the paracellular Cl^- transport, via phosphorylation of members of the claudin family that encode tight junction proteins. Transgenic

Figure 38-5

Genetic heterogeneity of Pseudohypoaldosteronism type II. Three loci have been identified on chromosome 1 (PHA2-A), chromosome 17 (PHA2-B) and latest on chromosome 12 (PHA2-C). Genes corresponding to two of these loci have been identified, *WNK4* for the PHA2-B and *WNK1* for the PHA2-C locus, respectively. Further genetic heterogeneity exists since several affected families do not show linkage at these loci (for details see text).

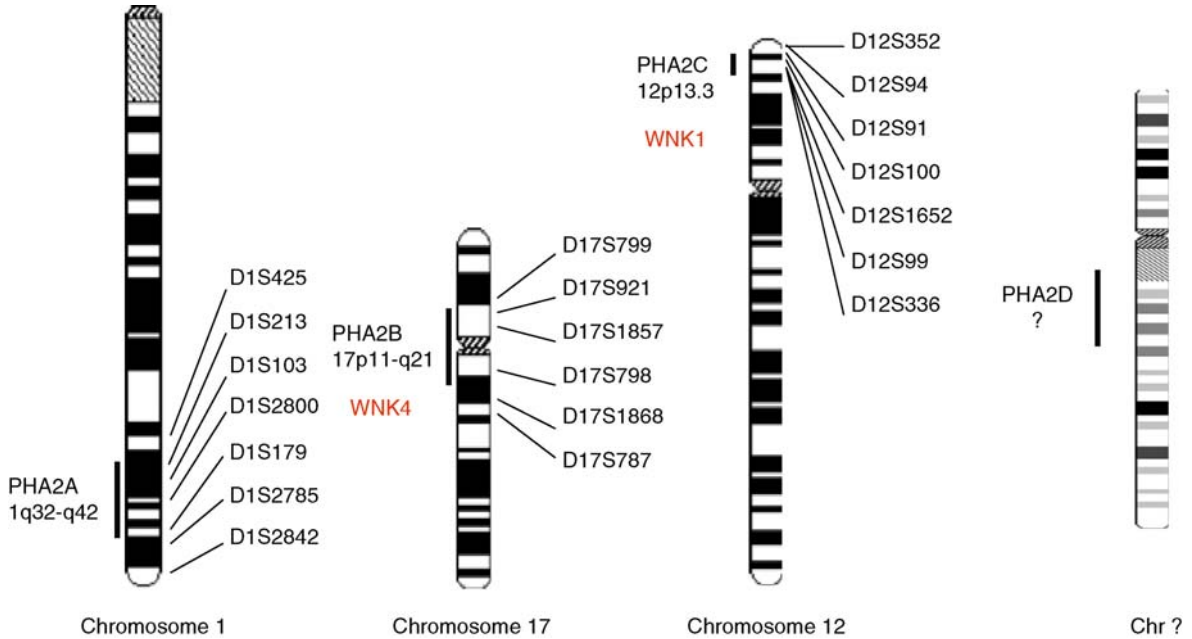
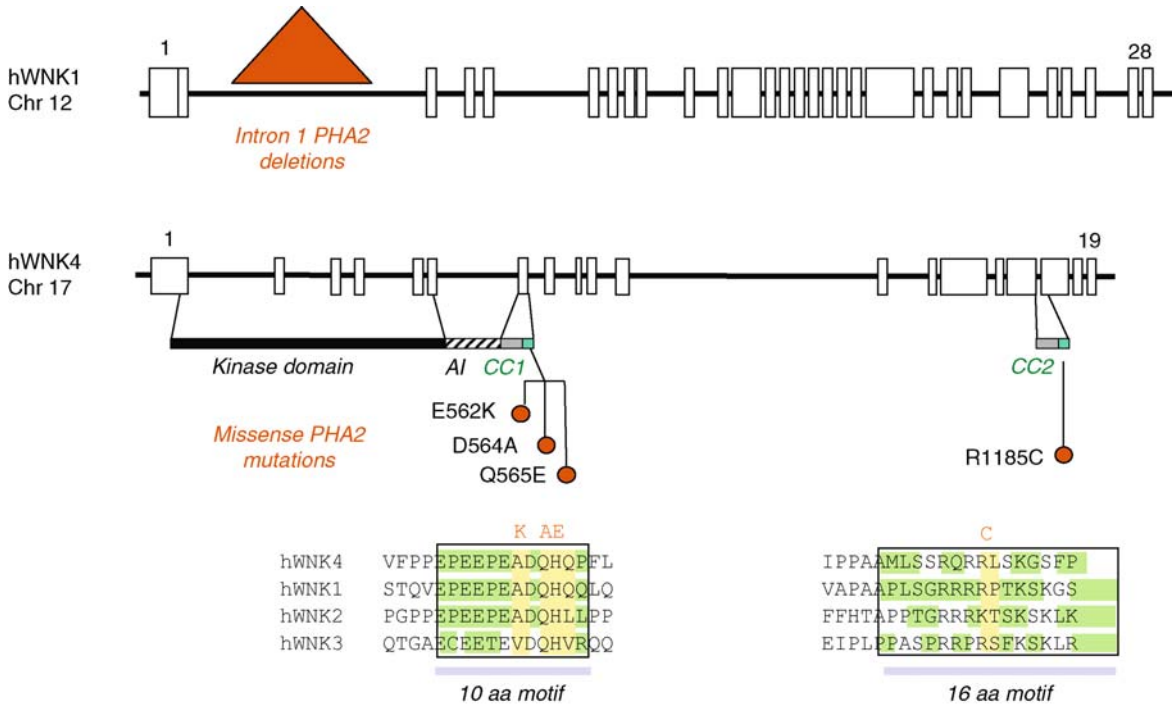


Figure 38-6

WNK1 and WNK4 mutations in pseudohypoaldosteronism type II. PHA2 causing mutations at the *WNK1* gene correspond to large (41 and 22 Kb) deletions in the intron 1. As a consequence of the suppression of important regulatory elements, the expression of the L-WNK1 and KS-WNK1 are increased in the renal tubule. PHA2 causing mutations at the *WNK4* gene correspond to missense mutations located into two conserved negatively and positively charged regions downstream the two coiled-coil domains.



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 (361, 362). Thus, WNK4 could be a determinant of the choice faced by the kidney between maximal NaCl reabsorption and K^+ secretion in response to aldosterone secretion and this determination could be mediated mainly by modulating NCCT activity.

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 (363). 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 Glucocorticod Kinase SGK1 and ENaC. Several studies in vitro have shown that WNK1 may act as an osmotic sensor in various cells (364). 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 (358).

It is not clear yet whether the two genetic defects on *WNK1* and *WNK4* give a 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 analysed for this parameter (350). In the WNK4 pedigree analysed by Farfel'group (365), affected members had hypercalciuria with normomagnesemia and decreased bone mineral density. No susceptibility to kidney stones was observed. Transgenic mice overexpressing a mutant WNK4 also display increased blood pressure, hyperkalemia, hypercalciuria together with marked

hyperplasia of the DCT (362). The effet on the calcium balance could be due to the interaction between WNK4 and TRPV4 which are coexpressed in the distal nephron (366).

Therapy

Low salt regimen can be in part effective in PHA2 as also evidenced in other forms of volume-dependent hypertension (367). Low-salt diet can be sufficient, especially in childhood, since blood pressure might be normal and chronic hyperkaliemia is usually well tolerated. Thiazides 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 daily) are very efficient to correct both biochemical abnormalities and blood pressure in a few weeks. Thiazide diuretics also correct the hypercalciuria that is observed in WNK4-linked PHA2 (365). Furosemide is also effective but less logical since it increases hypercalciuria and could enhance the risk of nephrolithiasis. From our experience, there is no loss of efficiency of thiazides along the years. However, their potential metabolic side effects constitute a rationale to develop new antihypertensive agents controlling the WNK pathway.

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 hyponatriemia, hyperkalemia and metabolic acidosis, despite extremely high values of plasma renin and aldosterone (368). 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 (369). There exist two different clinical forms of PHA1: (1) a renal form, in which mineralocorticoid resistance is restricted to the kidney, and (2) a generalized form, where mineralocorticoid resistance is systemic and salt loss occurs in multiple organs (370) (*Table 38-7*).

Clinical and Biochemical Features

Renal PHA1. Renal PHA1 (also called autosomal dominant PHA1) (371) is a mild and most frequent form of the disease. Clinical expression is variable: in general, patients show a neonatal salt losing syndrome, with weight loss, failure to thrive, vomiting and dehydration. Biological findings are hyponatremia, hyperkalemia and inappropriately high urinary Na^+ excretion, with occasional hypercalciuria. Urinary K^+ excretion is low, with reduced fractional K^+ excretion and transtubular K^+ gradient (372, 373). The diagnosis is confirmed in 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 Na^+ homeostasis in these patients are not clear; most likely, kidney maturation, access to dietary salt, compensatory increase in proximal Na^+ reabsorption as well as the up-regulation of the mineralocorticoid axis all play a role to compensate for the distal salt loss. Indeed, high plasma aldosterone levels persist into adulthood, while plasma renin activity decreases into normal range (370, 374, 375).

Generalized PHA1. In contrast to the renal form, patients affected by generalized PHA1 (also called autosomal recessive PHA1) (371) present with severe salt wasting from kidney, colon, sweat and salivary glands (376). In addition to severe dehydration, vomiting and failure to thrive, the clinical picture may be complicated by cardiac dysrhythmias, collapse, shock or cardiac arrest (374). Severe hyperkalemia and high aldosterone and plasma renin levels orient the diagnosis that can be completed by a positive salivary or sweat test. The prognosis of this form of PHA1 is poor: no remission has been reported and patients suffer from recurrent, life-threatening episodes of salt loss. In addition to the renal phenotype, frequent respiratory tract illnesses have been observed, evoking cystic fibrosis, that are caused by an increase in the volume of airway surface liquid (377). Also, an eczematoid rush of the skin is frequent in these patients, due to the severe salt loss from sweat glands (374, 378, and unpublished observations).

Transient pseudohypoaldosteronism has been observed in infants less than 7 months of age suffering from urinary tract malformations or urinary tract infections (379–381). In these patients, medical or surgical care of the primary disease restores the normal response to aldosterone. 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 ultrasonograpy and urine cultures in all children presenting with salt wasting and hyperkalemia (379). Secondary PHA1 may also develop in the adult following resection of the ileum and colon (382) or after renal transplantation (383).

Table 38-7

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 rush 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 Na^+	N	↑
Salivary Na^+	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, β, γ Subunits of the epithelial Na^+ channel ENaC

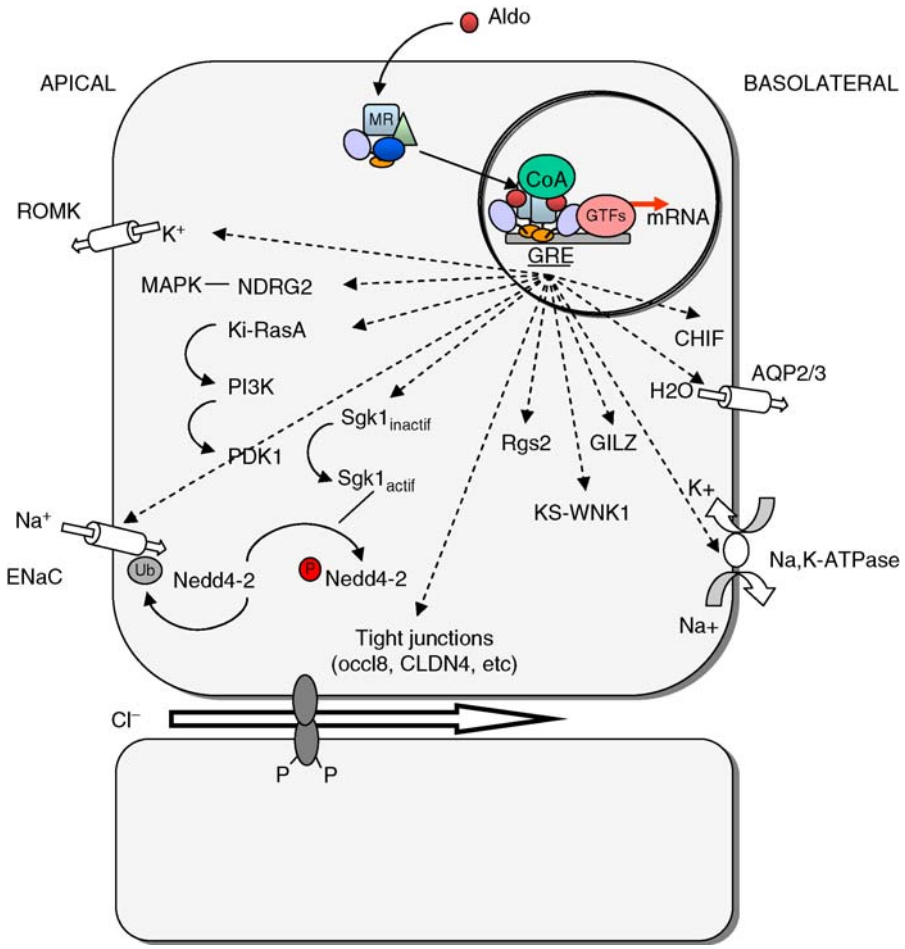
Pathophysiology and Genetics

Since the first description of PHA1, a defect of the tubular response to aldosterone had been suggested as the underlying cause. The mineralocorticoid receptor (MR) classically mediates aldosterone effects on electrolyte balance and blood pressure by regulating trans-epithelial sodium transport through tight epithelia (*Fig. 38-7*). The MR belongs to the nuclear receptor superfamily and acts as a ligand-activated transcription factor regulating expression of a coordinate set of genes involved in the physiologic response to aldosterone, including ENaC (384, 385). Armanini et al. confirmed the hypothesis of an abnormal MR-aldosterone axis by showing that ^{3}H-aldosterone binding was absent or very low in mononuclear leukocytes of affected patients (386). In a detailed study of 8 families, a dual pattern of inheritance was observed that correlated with receptor binding abnormalities. In some families an autosomal recessive inheritance 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 (387).

Subsequently it was shown that the two clinical forms of PHA1 are caused by different genetic defects. Inactivating mutations in the *MR* (or *NR3C2*) gene coding for the MR are found in renal PHA1 (368, 388, 389). Patients are

Figure 38-7

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 down-regulated gene 2 (NDRG2)) and others. Aldo, Aldosterone; apical and basolateral indicate the two poles of the epithelial cell.



always heterozygous for the mutations, which occur at high frequency both in patients with familial autosomal dominant PHA1 and patients with a sporadic renal presentation (390). In the latter group, only one third are de novo mutations, implying that carriers develop clinically evident disease only in a small proportion of kindreds. Although the exact causes for the variable phenotypic expression of renal PHA1 are unknown, possible reasons include the occurrence of events that trigger neonatal salt loss, such as intercurrent volume-depleting events or infections. Also, naturally occurring hypomorphic or hyperfunctioning alleles of other genes, coding for proteins involved in distal sodium reabsorption, may aggravate or attenuate the phenotype.

The severe and generalized, recessive form of PHA1 is due to mutations in the genes coding for the α, β and γ subunits of the sodium channel ENaC, *SCNN1A*, *SCNN1B*, and *SCNN1G*, respectively. Deleterious mutations have been found in affected patients being either homozygous – in consanguineous families – or composite heterozygous (385, 391–393). They include missense and nonsense mutations, deletions, insertions and splice site

junction mutations leading to abnormal mRNA splicing. Mutations appear in all ENaC subunits, but are more frequent in the α subunit, consistent with its determinant role in channel function. None of these mutations occur in the cytoplasmic C-terminus of ENaC subunits, where mutations result in a hyperfunctioning channel and a clinical phenotype, Liddle's syndrome, which is a mirror image of PHA1.

The pathogenic mechanism of PHA1 in patients with heterozygous MR mutations depends on the mutation. Although haploinsufficiency is sufficient to cause autosomal dominant PHA1 (371, 394), mutated receptors may also exert dominant negative effects on the wild type receptor (394), since the MR regulates 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 (395). In generalized PHA1, inactivating mutations affect one of the subunits of the amiloride-sensitive sodium channel ENaC. Absence of amiloride-sensitive Na^+ transport across airway epithelia has been evidenced in a neonate with generalized PHA1 by measuring transepithelial voltage across the nasal epithelium (396). The mutations causing generalized PHA1 are distributed all along the sequence of ENaC subunits. It is conceivable that nonsense, frameshift mutations, as well as mutations leading to abnormal splicing, cause a channel decrease or a loss of 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 (385).

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) (397, 398), replacement therapy with fludrocortisone and hydrocortisone may be undertaken while confirming the diagnosis by hormonal measurements. Early postnatal hyperkalemia may sometimes complicate aBS, due to mutation in the potassium channel ROMK (44). 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. In renal PHA1, 3–20 mEq/kg/day of NaCl, 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 Na^+ delivery to the distal nephron, stimulating K^+ secretion. Nevertheless, ion exchange resins are often included to the treatment to normalize plasma K^+ levels. The amount of Na^+ required is deduced from the normalization of plasma K^+ 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 psycho-motor development.

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 K^+ levels. Corticoid treatment is sometimes associated and seems to provide some additional benefit. Administration of indomethacin may be useful in occasional patients (399). 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 (374, 375).

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