The Kidney in Genetic and Rare Diseases: Review

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Genetics of Magnesium Disorders

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Abstract
Background: Magnesium (Mg^{2+}), the second most abundant cation in the cell, is woven into a multitude of cellular functions. Dysmagnesemia is associated with multiple diseases and, when severe, can be life-threatening. ***Summary:*** This review discusses Mg^{2+} homeostasis and function with specific focus on renal Mg^{2+} handling. Intrarenal channels and transporters related to Mg^{2+} absorption are discussed. Unraveling the rare genetic diseases with manifestations of dysmagnesemia has greatly increased our understanding of the complex and intricate regulatory network in the kidney, specifically, functions of tight junction proteins including claudin-14, -16, -19, and -10; apical ion channels including: TRPM6, K_v1.1, and ROMK; small regulatory proteins including AC3 and ANK3; and basolateral proteins including EGF receptor, γ-subunit (*FXYD2*) of Na-K-ATPase, K_{ir}4.1, CaSR, CNNM2, and SLC41A. Although our understanding of Mg^{2+} handling of the kidney has expanded considerably in the last two decades, many questions remain. Future studies are needed to elucidate a multitude of unknown aspects of Mg^{2+} handling in the kidney. ***Key Message:*** Understanding rare and genetic diseases of Mg^{2+} dysregulation has expanded our knowledge and furthers the development of strategies for preventing and managing dysmagnesemia.

Introduction

Magnesium (Mg^{2+}) is the second most abundant intracellular cation. It carries out multiple and critical functions supporting cellular physiological activities. Sixty percent of the US population, however, show insufficient Mg^{2+} intake [1], and hypomagnesemia occurs in ~30% of hospitalized patients [2]. The kidney is the principle organ that regulates Mg^{2+} homeostasis. In the last two decades, a number of new Mg^{2+} regulatory mechanisms have been uncovered, mainly through investigating patients with rare genetic alterations as the cause of Mg^{2+}

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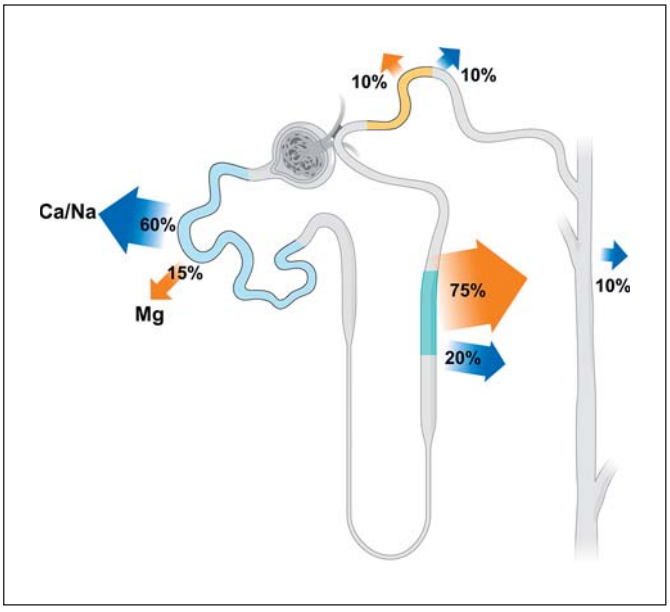

Fig. 1. Renal tubular Mg^{2+} handling. More than 95% of the filtered Mg^{2+} is reabsorbed in the kidney, and only approximately 100 mg of Mg^{2+} is excreted daily. The distal convoluted tubule is the last part where Mg^{2+} reabsorption occurs. Beyond that section, the kidney tubules are impermeable to Mg^{2+}.

dysregulation. This review focuses on recent advances in our understanding of Mg^{2+} regulation in the kidney and dysregulation in patients with specific genetic disorders and poses questions on a number of unresolved issues in this field.

Magnesium Homeostasis and Function

The medicinal function of Mg^{2+} was recognized as early as 1618, when in Epsom, England, a farmer realized that his bitter salty well water healed scratches. "Epsom salts" (primarily Mg^{2+} sulfate) can be found today in any big-box store such as Walmart or Costco and are often used as a bath salt and a remedy for relieving many ailments including joint pain, muscle spasms, abdominal pain, constipation, headaches, and more. Over the years, studies have shown that Mg^{2+} is a vitally important, indeed a critical, intracellular element. It is a cofactor/coactivator for more than 600 intracellular enzymes and an essential component of DNA replication, RNA transcription, amino acid synthesis, and protein formation. It is also critical and participates in DNA repair including nucleotide excision repair, base excision repair, and mismatch repair. Lack of the inner mitochondrial membrane Mg^{2+} channel, Mrs2, leads to respiratory complex I failure and cell death [3]. Additionally, Mg^{2+} has anti-inflammatory, immunomodulatory, and crystal-inhibitory properties [4]. In the last two decades, genome-wide association studies have shown that polymorphisms in genes related to Mg^{2+} homeostasis are associated with multiple diseases or risks for diseases including diabetes in African-American and Spanish-American postmenopausal women [1], nephrolithiasis, and reduced bone mineral density [5]. Recently, *TRPM7* gene polymorphism has been linked to breast cancer [6]. In addition, studies have shown that Mg^{2+} alterations can be associated with neurotransmitter defects [7, 8] and an array of neurological abnormalities [9, 10].

Dietary Mg^{2+} intake in the general adult population should be in the range of 350–450 mg/day. Fecal excretion of Mg^{2+} is ~270 mg/day. Mg^{2+} is absorbed from the gastrointestinal tract, through paracellular transport in the small intestine and transcellular transport in the colon, into the blood, where ~25% of the absorbed Mg^{2+} binds to circulating proteins, primarily albumin. The unbound Mg^{2+} equilibrates with bone and intracellular Mg^{2+}. Intracellular Mg^{2+} concentrations are in the range of 10–30 mM, and the free cytosolic Mg^{2+} concentration is ~0.5–1.2 mM [11]. Approximately 70–75% of the circulating Mg^{2+} is unbound and is filtered in the kidneys. The glomeruli filter ~2,400 mg of Mg^{2+} daily. More than 95% of the filtered Mg^{2+} is absorbed, leaving a urinary Mg^{2+} excretion of ~100 mg/day. Predictably, Mg^{2+} is regulated principally by the kidneys.

Magnesium Absorption in the Proximal Convoluted Tubule

In contrast to the proximal absorption of calcium (Ca^{2+}) and sodium (Na^{+}), a relatively small fraction (15%) of filtered Mg^{2+} is absorbed in the proximal tubules (Fig. 1) via a paracellular mechanism, although the precise mechanism has not been fully elucidated. In the first portion of the proximal convoluted tubule, Na^{+}, Ca^{2+}, and water are absorbed to a large extent, preceding Mg^{2+} absorption. When the filtrates reach more distal parts of the proximal tubule, there is an increase in tubular fluid Mg^{2+} concentration compared to that in the peritubular circulation. When the concentration gradient of Mg^{2+} between the luminal fluids and the peritubular circulation reaches approximately 1.7–1.9, Mg^{2+} absorption occurs, and is likely driven by the concentration gradient [12].

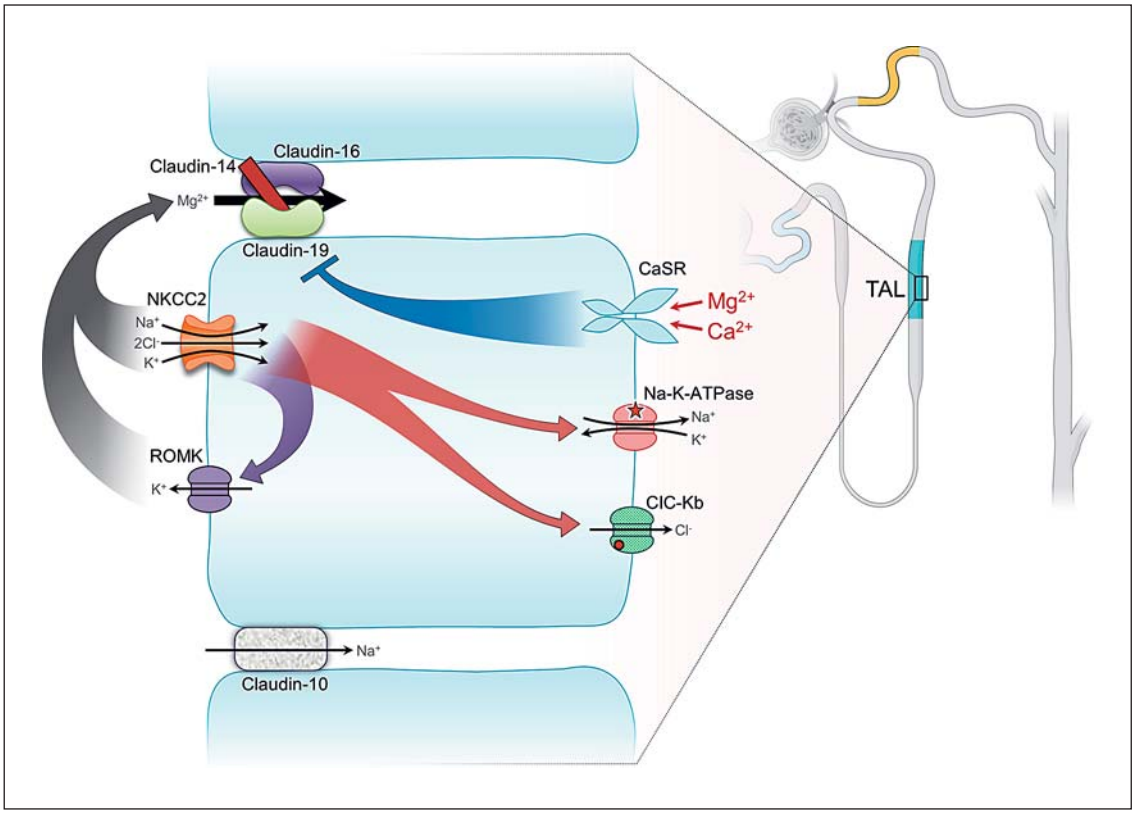

Fig. 2. Mg^{2+} handling in the thick ascending limb of Henle (TAL). The tight junction proteins claudin-16 and -19 form the Ca^{2+}- and Mg^{2+}-permeable channel. Mg^{2+} or Ca^{2+} in the circulation could activate the basolateral calcium-sensing receptor (CaSR), which exerts inhibitory effects on the tight junction claudin complex. Basolateral Na-K-ATPase provides the driving force for the apical Na-K-2Cl cotransporter (NKCC2) and parallel K^+ excretion via ROMK, generating a favorable positive luminal voltage to facilitate paracellular Mg^{2+} and Ca^{2+} absorption.

Magnesium Absorption in the Thick Ascending Limb of Henle

In the thick ascending limb of Henle (TAL), Mg^{2+} is absorbed paracellularly, facilitated by tight junctional proteins, and the major driving force is the transepithelial voltage gradient. In the initial part of the TAL, the luminal voltage is positive (approx. +8 mV), and the Na^+ and Cl^- concentrations are relatively high (Fig. 2). The positive voltage is generated primarily through the apical Na-K-2Cl cotransporter (NKCC2)-mediated Na^+, K^+, and Cl^- absorption with parallel K^+ excretion to the lumen. The driving force for the NKCC2 is the basolateral Na-K-ATPase. The positive transepithelial voltage gradient propels Mg^{2+} absorption via paracellular mechanisms. At the distal end of the TAL, where the Na^+ concentration diminishes, Mg^{2+} absorption is further regulated by tight junction proteins between the adjacent tubular epithelial cells, specifically claudin-16, -19, and -14 [13].

Claudins

Claudins (*CLDN*) are small tetraspan proteins (MW: 20–28 kDa) composing a family with at least 26 members. They are key components in the formation of the tight junction barrier and responsible for regulated paracellular ion flux [13]. In the TAL, paracellular Mg^{2+} absorption is facilitated by the combined effect of claudin-16 (paracellin-1) and claudin-19, and is inhibited by claudin-14 [14–17] (Fig. 2). Such arrangements represent a regulatory mechanism for controlled Mg^{2+} absorption. Studies have shown that the basolateral calcium-sensing receptor (CaSR), when activated, increases the expression of claudin-14, preventing excess Mg^{2+}/Ca^{2+} absorption, responding to changes of the extracellular Ca^{2+} or Mg^{2+} concentration (detailed below).

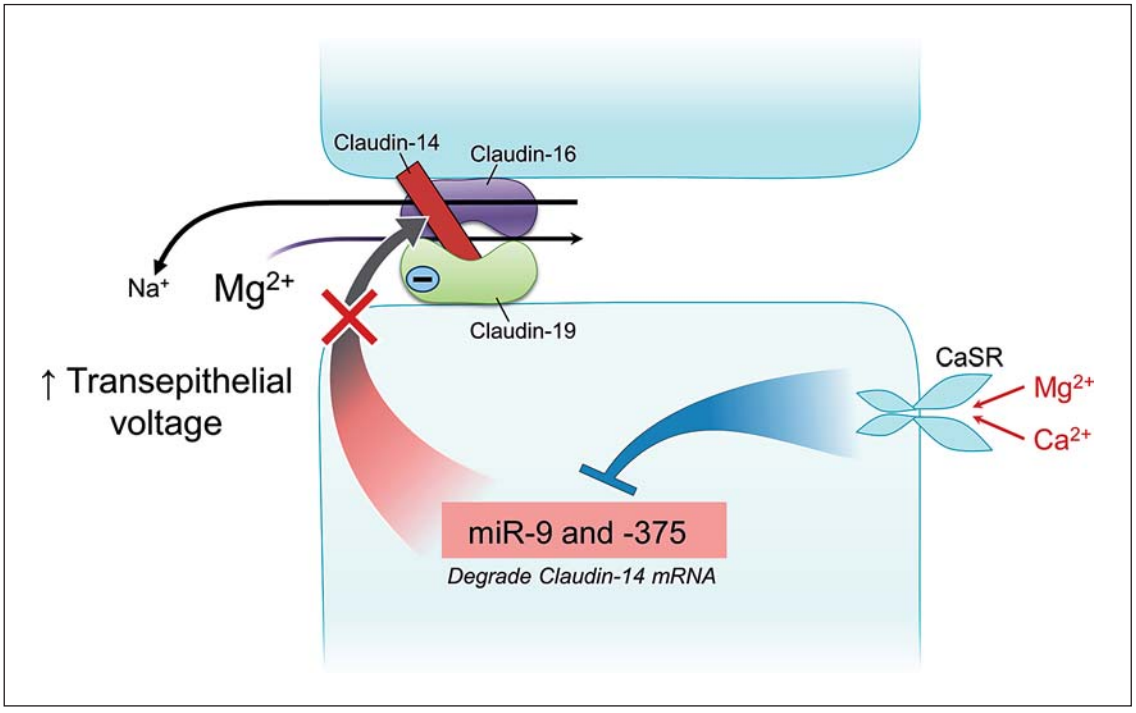

Fig. 3. Regulated paracellular Mg^{2+} absorption in the thick ascending limb of Henle (TAL). Claudin-16 by itself is highly permeable to Na^+, and claudin-19 is impermeable to Cl^-. Claudin-14 deters the sodium channel permeability of claudin-16. Na^+ backleaks into the distal part of the TAL and helps in maintaining a positive luminal voltage. Blockage of this action of claudin-14 compromises the positive luminal voltage and diminishes the driving force for Mg^{2+} absorption. The deterrence of Cl^- passage by claudin-19 creates a negative microdomain charge, creating a selective attraction to luminal Mg^{2+} and Ca^{2+}. The basolateral calcium-sensing receptor (CaSR), when activated by an elevated concentration of Mg^{2+} or Ca^{2+}, inhibits miR-9 and miR-375. The inhibition removes the interference of the microRNAs with claudin-14 translation, resulting in an increased claudin-14 translation and claudin-14-mediated inhibition of claudin-16, thus inhibiting Mg^{2+} and Ca^{2+} absorption.

In the last two decades, studies have elucidated underlying mechanisms in more detail. Claudin-16 and -19 colocalize in the TAL to form a cation complex [16]. Claudin-16 by itself is an Na^+-permeable channel [18], and claudin-19 is a deterrent protein to the passage of Cl [16]. The Cl deterrence by claudin-19 creates a negative microenvironment in the epithelial junction, creating an attraction for cation (Mg^{2+}/Ca^{2+}) selection. At the end of the TAL, where the luminal Na^+ concentration is low and the basolateral Na^+ concentration relatively high (approx. 140 mM), through claudin-16 there is a backleak of Na^+ from the basolateral aspect to the lumen. Such a backleak enhances the positive luminal voltage, fostering paracellular Mg^{2+} absorption (Fig. 3).

Recently, a claudin-10 (*CLDN10*) knockout mouse model has shown that claudin-10 [19], which is expressed almost exclusively in the tubular junction of the epithelia in the TAL, functions as an Na^+-permeable protein. Deletion of claudin-10 would deter Na^+ absorption and create an elevated luminal transepithelial voltage to increase Mg^{2+} absorption. Animals with claudin-10 deletion show elevated serum Mg^{2+} and Ca^{2+} concentrations and an impaired capacity of urine Mg^{2+} and Ca^{2+} excretion.

Calcium-Sensing Receptor

CaSR, a member of the G protein-coupled receptor superfamily, is a 120-kDa polypeptide containing seven transmembrane domains. CaSR is expressed abundantly in parathyroid glands and in kidney tubules. It also is expressed in multiple organ systems including the cardiovascular, gastrointestinal (specifically the ileum), airway, and central nervous systems [20]. Recent studies have provided compelling evidence that by recognizing and responding to miR-9 and miR-374 (small noncoding RNA molecules) [17], CaSR regulates the expression of claudin-14 in the TAL (Fig. 3), thereby regulating paracellular Mg^{2+} absorption. Recessive mutations in claudin-14 have been reported as a cause of nonsyndromic recessive deaf-

Table 1. Dysmagnesemia related to salt-losing nephropathy

	Inheritance	Mutant gene	Transporter/ protein	Key features
Bartter syndrome I	AR	*SLC12A1*	NKCC	Regulated by CaSR, antenatal onset, hypokalemic, hypercalciuric metabolic alkalosis
Bartter syndrome II	AR	*KCNJ1*	ROMK	Antenatal onset, can be hyperkalemic
Bartter syndrome III	AR	*ClC-Kb*	ClC subunit B	Variable childhood onset; milder form of Bartter syndrome; variable, Gitelman-like presentation
Bartter syndrome IV	AR	*BSND*	Barttin	Sensorineural hearing defect, CKD/ESRD in second or third decade of age
Bartter syndrome V	AD	*CASR* (activating mutation)	CaSR	Neonatal onset, can be variable; urine Mg^{2+} and Ca^{2+} loss
Gitelman syndrome	AR	*SLC12A3*	NCC	Childhood and adolescent onset, hypocalciuric hypokalemic, metabolic alkalosis, urine Mg^{2+} loss

AR, autosomal recessive; AD, autosomal dominant; NKCC, Na-K-Cl cotransporter; CaSR, calcium-sensing receptor; NCC, Na-Cl cotransporter; CKD, chronic kidney disease; ESRD, end-stage renal disease.

ness (DFNB29) [21] due to failure of ion balance in the organ of Corti [22]; there is no information as to any renal manifestations in affected individuals.

The importance of CaSR in the regulation of Ca^{2+} and Mg^{2+} has been demonstrated in mice models of $Casr^{+/-}$ and $Casr^{-/-}$ mutations. The mutant mice phenocopy the manifestations of humans with familial hypocalciuric hypercalcemia (FHH) and neonatal severe hyperparathyroidism (NSHPT). Consistently, the extracellular Mg^{2+} concentration is moderately elevated in $Casr^{+/-}$ mice, and more severely elevated in $Casr^{-/-}$ mice [23].

Additional Transporters

NKCC, ROMK, ClC-Kb, and barttin all contribute to a positive transepithelial voltage, favoring Mg^{2+} absorption. ROMK, ClC-Kb, and barttin cross the boundary of TAL and are expressed in the distal convoluted tubule (DCT). They have been described in detail in the context of salt-losing nephropathy (Table 1) [24–29].

Dysmagnesemia due to Ion Channel/Transporter Mutations in the TAL

Hypercalciuria and Nephrocalcinosis

CLDN16 and *CLDN19* mutations are responsible for familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) [14, 15]. FHHNC was initially reported in 1997 by Walder et al. [30] in three consanguineous Bedouin kindreds from Israel. *CLDN16* mutations were found to be causative. Affected individuals exhibit severe and symptomatic hypomagnesemia in the range of 0.1–0.4 mM, hypercalciuria, and a nephrocalcinosis onset at the age of 2–8 weeks after birth. The symptoms include polyuria/polydipsia, muscular tetany, seizures, nephrolithiasis, and progressive loss of kidney function. Renal failure develops mostly in childhood or in adolescence [31]. In 2006, Konrad et al. [15] reported initial cases of FHHNC due to *CLDN19* mutation – but without *CLDN16* mutation – in Swiss and Spanish families, with nearly identical presentations. Patients with *CLDN19* mutations additionally show ocular abnormalities. Recent studies have shown that both *CLDN16* and *CLDN19* mutations can give rise to abnormal enamel formations [32, 33].

More information is emerging on the spectrum of mutations, primarily in *CLDN16*, since approximately 46 mutations have been described to date, as well as the genotype-phenotype correlations. In a study on 25 families with FHHNC in Germany and Eastern European countries, a founder mutation (L151F) in the first extracellular loop of claudin-16 has been identified as being present in about 50% of mutant alleles [31]. Patients with loss-of-function mutations in both alleles in the *CLDN16* gene tend to have an early onset of renal failure, whereas those harboring at least one mutant allele with some residual function seem protected from the rapid loss of renal func-

tion [34]. Interestingly, a novel homozygous *CLDN16* mutation (T233R) has been identified in two families with self-limiting childhood hypercalciuria. The hypercalciuria decreased with age and was not associated with declined renal function. The T233R mutation is the first mutation to be identified in the cytosolic tail of claudin-16, and is predicted to result in an ineffective PDZ domain-binding motif. This inactivation abolishes the association with the tight junction scaffolding protein ZO-1, with subsequent accumulation of the mutant claudin-16 protein in lysosomes [35]. The underlying mechanism for such a mild phenotype in patients with this mutation is unknown. Much less is known about the genotype-phenotype correlations in *CLDN19* mutations. Indeed, to date 14 mutations have been described, and no correlation between *CLDN19* mutations and the rate of renal functional deterioration has been uncovered [15, 33, 36].

Autosomal Dominant Hypocalcemia and FHH

CASR mutations cause a spectrum of phenotypes. Gain-of-function mutations are causative of autosomal dominant hypocalcemia (ADH). Loss-of-function mutations cause autosomal dominant FHH and autosomal recessive (homozygous or compound heterozygous mutations) NSHPT. Patients with gain-of-function *CASR* mutations develop varying degrees of hypocalcemia and hypomagnesemia from a stable and often mild form of ADH to a severe form of Bartter syndrome type V (Table 1). Opposite abnormalities of hypercalcemia and hypermagnesemia occur in patients with loss-of-function mutations. Most FHH patients are clinically asymptomatic and exhibit mild hypercalcemia and often hypermagnesemia, while NSHPT patients are characterized by severe hypercalcemia and typically die within 1 year after birth if parathyroidectomy is not provided [37].

Considerable clinical heterogeneity has been observed among patients with heterozygous *CASR* mutations. A small number of affected patients develop neonatal hyperparathyroidism associated with mild-to-moderate hypercalcemia during infancy. It was formerly assumed that a dominant negative effect and/or paternal inheritance could have modified the clinical presentation. Recent functional studies, however, have found no evidence for either effect [38], suggesting that other environmental or epigenetic factors yet to be identified might have played a role in the various disease presentations. Over 230 *CASR* mutations have been described, and several mutational hot spots and genotype-phenotype correlations of some degree have been demonstrated. For instance, functional studies of several mutations have shown that mutations in C131W and A843E preferentially alter the Ca^{2+} response curve, differing from other *CASR* mutations (IEK47N and P221L) without clinical features of urine Mg^{2+} wasting and hypomagnesemia [39]. More comprehensive information regarding *CASR* mutations and clinical manifestations have recently been comprehensively reviewed [40].

Magnesium Absorption in the DCT

The last approximately 10% of Mg^{2+} are absorbed in the DCT. Mg^{2+} absorption in this tubular section is transcellular instead of paracellular. The Mg^{2+} absorption is tightly regulated through multitudes of channels and transporter proteins. The more stringent regulation is justly called for, since this is the last part of the renal tubules with the capacity for absorbing luminal Mg^{2+}. It fine-tunes and determines the final amount of urine Mg^{2+} excretion. The chemical gradient of Mg^{2+} in this section is small (luminal Mg^{2+} concentration: 0.2–0.7 mM; intracellular free Mg^{2+}: approx. 0.5–1.2 mM). Thus, a positive transapical membrane voltage gradient becomes critical for Mg^{2+} entry. All known regulatory pathways converge onto influencing the transapical membrane voltage, the principle driving force for Mg^{2+} entry into the DCT cells via TRPM6 (Fig. 4). The function and regulation of some transporters, e.g., SLC41A and CNNM2, have yet to be fully elucidated.

TRPM6

Two groups independently identified TRPM6 as the causative gene in hypomagnesemia with secondary hypocalcemia (HSH) in 2002 [41, 42]. TRPM6 is a 234-kDa membrane protein with both its N- and its C-termini residing in the cytosol. It has six transmembrane domains with a channel function between transmembrane domains 5 and 6. It has an approximately 5-fold higher affinity for Mg^{2+} than for Ca^{2+} [43]. Accordingly, patch clamp analysis showed that TRPM6 is an Mg^{2+}- and Ca-permeable cation channel preferentially transporting Mg^{2+} [43]. TRPM6 proteins form homotetrameric functional complexes as well as heterotetrameric complexes with TRPM7, the closest homolog of TRPM6 [44]. Both TRPM7-dependent and TRPM7-independent TRPM6 activity have been reported [45, 46].

TRPM6 contains multiple interacting regions. For instance, around channel areas, there is a TRP box that interacts with PIP_2. Immediately downstream of the channel domain there is a coiled-coiled region that is believed to form dimers with other TRPM monomers. Insulin can

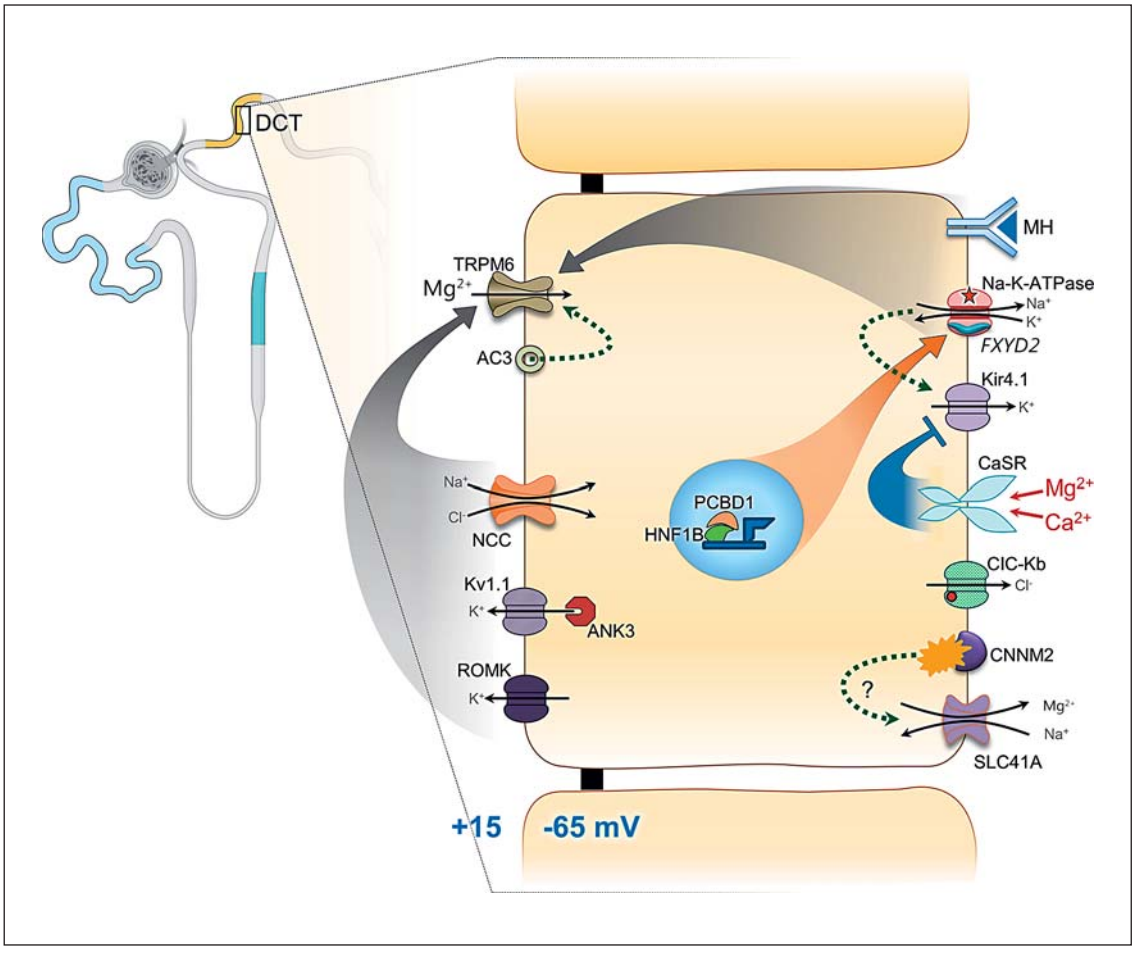

Fig. 4. Schematic presentation of magnesium regulation in the distal convoluted tubule. TRPM6 is the major channel for Mg^{2+} absorption in the distal convoluted tubule. The driving force for TRPM6-mediated Mg^{2+} intake is a positive apical membrane potential gradient. Na-K-ATPase is critical in the establishment of the transapical membrane gradient. PCBD1 and HNF1B are transcription factors promoting the expression of the γ-subunit of the Na-K-ATPase, encoded by *FXYD2*. Mutations in *HNF1B* and *PCBD1* interfere with transcriptional expression of the γ-subunit, thus leading to an insufficient transapical membrane potential and diminishing Mg^{2+} absorption via TRPM6. Channels important for the establishment of the apical membrane potential include transporters for K^+ excretion apically, $K_v1.1$, and ROMK. Calcium-sensing receptor (CaSR) inhibits basolateral K^+ exit via $K_{ir}4.1$. Reduced K^+ recycling via $K_{ir}4.1$ interferes with Na-K-ATPase function. ClC-Kb is a basolateral Cl^- exit channel. Its mutation and the mutation of its subunit barttin could both cause defects in Cl^- exit in association with Na-Cl cotransporter (NCC) function and the apical membrane potential. CNNM2 is a basolateral Mg^{2+}-Na^+ exchanger which may function as a cytosolic Mg^{2+} sensor. SLC41A encodes an Mg^{2+} exit channel. The regulation(s) and physiological function(s) of the channels of CNNM2 and SLC41A are yet to be elucidated. MH, magnesiotropic hormones (including EGF and insulin).

react in the Ser-Thr-rich region upstream adjacent to the kinase domain. In the C terminus, there is an α-kinase domain that plays a regulatory role in response to RACK-1 and REA. These regions are all targets of potential signaling interactions [47, 48]. Thus, it comes as no surprise that TRPM6 can be activated by a number of signals including EGF, insulin, estrogen, purinergic signaling, dietary Mg^{2+} intake, and acid-base alterations. Recent studies have shown that plasma membrane TRPM6 expression can be stimulated by the adenylyl cyclase 3 (AC3)/cAMP/PKA-mediated signaling pathway. AC3 is also colocalized with Na-Cl cotransporter (NCC) in the DCL [49]. In mice with kidney-specific AC3 knockout, urine Mg^{2+} wasting, hypomagnesemia when on a low-Mg^{2+} diet, colocalization of AC3 and NCC, and activation of cAMP-PKA with increasing apical membrane expression and channel conductance of TRPM6 have all been demonstrated [49].

Pro-EGF/EGF and EFG Receptor

Pro-EGF is expressed in the renal epithelia, primarily in the DCT [50, 51]. Pro-EGF is proteolytically cleaved, and functional EGF is released. The released EGF activates EGF receptor that is expressed at the basolateral aspect of the renal epithelial cells [52]. Receptor activation triggers a signaling cascade involving Src kinase and Rac1, leading to increased trafficking of TRPM6 from intracellular vesicles to the apical membrane to mediate Mg^{2+} absorption [53].

Gamma-Subunit of Na-K-ATPase

The γ-subunit of Na-K-ATPase, encoded by *FXYD2*, is critical for the pump function, as it stabilizes the α-subunit of this pump [54] and increases the pump's affinity for ATP while reducing its affinity for Na^+ and K^+. In the kidney, two splice forms of *FXYD2* genes are expressed: *FXYD2a*, expressed primarily in the TAL and proximal tubules, and *FXYD2b*, expressed exclusively in the basolateral membrane of the DCT and collecting duct [55]. A mutation in *FXYD2* causes misrouting of the mutant γ-subunit and fails to join the α- and β-subunits to form a complete and fully functional Na-K-ATPase [56]. The impairment of Na-K-ATPase function diminishes the driving force for apical NCC activity and compromises apical membrane voltage generation (partial depolarization of the cell), reducing the driving force for TRPM6-mediated Mg^{2+} entry. Reduced NCC activity also compromises TRPM6 activation associated with AC3/cAMP/PKA signaling [49].

HNF1B and PCBD1

HNF1B (hepatocyte nuclear factor 1 homeobox B) and PCBD1 (pterin-4α-carbinolamine dehydratase/dimerization cofactor of hepatocyte nuclear factor 1 homeobox A) are the two known transcriptional regulatory factors for *FXYD2* expression, especially in the distal tubules [57, 58]. HNF1B is involved in organogenesis and the formation of tubular structures in the liver, pancreas, lung, and kidney, and its expression seems restricted to epithelial cells [59, 60]. It has also been shown to transcriptionally activate *PKHD1*, the gene which, when mutated, causes autosomal recessive cystic kidney disease [61]. Indeed, mutations in *HNF1B* are emerging as the most frequent monogenic cause of developmental renal abnormalities [62]. PCBD1 is a dimerization cofactor for HNF1B in the nucleus [63, 64], as well as a cytosolic enzyme involved in the regeneration of tetrahydrobiopterin (BH_4) [65, 66]. Gene expression studies combined with immunohistochemical analyses of the kidney have shown that *Pcbd1* is highly expressed in the DCT, where *Pcbd1* transcript levels are upregulated by a low-Mg^{2+} diet [58, 67]. PCBD1 increases HNF1B-induced *FXYD2* transcription by 50% [58]. Mutant PCBD1 proteins were not capable of increasing *FXYD2* transcription [58].

KCNJ10

$K_{ir}4.1$ (potassium voltage-gated channel subfamily J member 10), encoded by *KCNJ10*, is expressed in the basolateral membrane of the DCT [68, 69]. $K_{ir}4.1$ mutation interferes with basolateral K^+ recycling, compromising Na-K-ATPase function and generation of the apical membrane potential. Convincing evidence has shown that CaSR, coexpressed in the same region, inhibits cell surface expression of $K_{ir}4.1$ [70] via a mechanism dependent on G_q and caveolin-1 [70, 71]. Reduced Na-K-ATPase activity due to $K_{ir}4.1$ mutations causes a reduced apical NCC activity and apical membrane voltage, the major driving force for Mg^{2+} influx [72]. Importantly, CaSR is able to modulate $K_{ir}4.1$-mediated potassium extrusion in response to the physiological range of the extracellular Ca^{2+} concentration (EC_{50} Ca^{2+} of 1.0 mM) [70]. Thus, through CaSR, $K_{ir}4.1$ regulates the distal nephron NaCl transport and apical membrane potential physiologically. Interestingly, in contradiction to classic teaching, no measurable CaSR-mediated ROMK inhibition could be demonstrated [70].

KCNA1

$K_v1.1$ (potassium voltage-gated channel subfamily A member 1), encoded by *KCNA1* [73], is an apical membrane K^+ channel contributing to the sustained transapical membrane voltage by extruding K^+ into the lumen. A mutation in $K_v1.1$ was identified as a causative factor for isolated autosomal dominant hypomagnesemia due to renal Mg^{2+} wasting [73]. A recent study by San-Cristobal et al. [74] showed that the function of $K_v1.1$ can be regulated by ankyrin-3 (ANK3), a member of adaptor proteins that link the cytoskeletal network to the cytoplasmic domain of plasma membrane proteins [75]. Mice on a high-Mg^{2+} diet have been shown to double their fractional urine Mg^{2+} excretion, associated with a 1.8-fold increase in renal ANK3 expression. Using the carboxy terminal domain of $K_v1.1$ to screen murine kidney lysates revealed that $K_v1.1$ function can be inhibited by ANK3. Biophysical studies have shown that ANK3 is able to functionally block the channel opening of $K_v1.1$ without affecting its plasma membrane expression. Thus, $K_v1.1$ is involved in establishing a positive and favorable electrical

apical membrane gradient to drive Mg^{2+} entry through TRPM6 in the epithelial cells of the DCT.

Notably, the ANK3-binding motif is not present in the $K_v1.1$ channel. Whether ANK3 binds the $K_v1.1$ channel directly or through other proteins in a macromolecular complex is currently not known. Nonetheless, the data clearly demonstrate a role for ANK3 in regulating the biophysical properties of $K_v1.1$, contributing to a physiologically relevant Mg^{2+} regulatory pathway.

CNNM2 and SLC41A3

The exact mechanism of basolateral Mg^{2+} efflux from the renal tubular cells is currently unknown. Studies have suggested that SLC41A3 [76, 77] and cyclin M2 (CNNM2) [78] may act as Mg^{2+} efflux channels. CNNM2 is also expressed in the basolateral membrane of the TAL [78]. CNNM2 contains two highly conserved CBS (cystathionine-β-synthase) domains, critical for dimerization with other transporters and also for the Mg^{2+}-dependent gating of the magnesium transporter E channel [79], a homolog of the SLC41 family of transporters [80]. Protein topology and homology modeling of a conserved CBS domain, however, suggest that CNNM2 might function as a cytoplasmic Mg^{2+} sensor [81–83]. The mechanism regulating the functions of CNNM2 and SLC41A has yet to be fully elucidated.

Other Transporters Affecting Mg^{2+} Homeostasis

Mutations in the genes encoding NCC, ROMK, ClC-Kb, and barttin (a subunit of ClC-Kb) have been characterized in more detail under the category of salt-losing nephropathies [84–90] (Table 1).

Dysmagnesemia due to Transporter/Protein Mutations in the DCT

Hypomagnesemia with Secondary Hypocalcemia

TRPM6 mutations are responsible for HSH, also known as primary intestinal hypomagnesemia. HSH patients typically present during the first few months of life with neurological symptoms of tetany and seizures associated with profound hypomagnesemia and secondary hypocalcemia due to parathyroid failure. HSH is associated with the most severe hypomagnesemia among all genetic forms of channelopathy, in the range of 0.05–0.20 mM in a recent study [48], due to the dual defects in gastrointestinal and renal Mg^{2+} absorption. To date, fewer than 50 unique mutations in *TRPM6* have been recorded [48]. Most of the mutations are predicted to give rise to a premature termination of *TRPM6* in approximately 85% of the mutations tested. The remaining mutations include missense mutations and mutations affecting the TRPM gating property or interfering with its plasma membrane trafficking. Thus far, most *TRPM6* mutant products tested in vitro in cellular systems have shown a dramatic decrease in channel currents, with the exception of the Q1663R variant, which functions almost the same way as the wild-type *TRPM6* [48]. Overall, there has been no clear pattern of genotype-phenotype association. It should be noted that in a number of cases, the diagnosis and consequent Mg^{2+} administration were delayed because the Mg^{2+} level was not measured on initial presentation, and the patients suffered from repeated convulsions leading to permanent brain damage and mental retardation [30, 41, 42].

Isolated Recessive Hypomagnesemia

Isolated recessive hypomagnesemia is an autosomal recessive disorder [91] caused by a mutation in the *EGF* gene, c.3209C>T (p.Pro1070Leu), causing a defect in the basolateral trafficking of pro-EGF, in turn leading to a defect in soluble EGF elaboration and in EGF-mediated apical TRPM6 expression [50]. Affected individuals present with seizures during infancy and mental retardation due to severe hypomagnesemia. Ca^{2+} derangement is typically spared [91]. Similarly, cetuximab, an EGFR antibody used as an anticancer agent, has been associated with severe hypomagnesemia due to lack of EGF receptor-mediated TRPM6 trafficking to the apical membrane and lack of Mg^{2+} absorption through TRPM6 [92, 93].

EAST Syndrome and SeSAME Syndrome

Mutations in the *KCNJ10* gene, encoding $K_{ir}4.1$, were shown to cause a syndrome named EAST (epilepsy, ataxia, sensorineural deafness, and tubulopathy) and SeSAME (seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance) by two independent groups [69, 94]. The syndrome is characterized by seizures, sensorineural deafness, and ataxia, as well as by electrolyte alterations akin to those in Gitelman syndrome including salt wasting, hypokalemic metabolic alkalosis, and hypomagnesemia. The serum Ca^{2+} concentration, however, tends to be reduced [69, 94]. Because mental retardation has not been a clinical feature, the term "EAST syndrome" seems appropriate and is used clinically. $K_{ir}4.1$ mutations, by interfering with Na-K-ATPase activity, compromise apical Na^+ entry via the NCC channel, thus partially depolarizing the apical membrane and diminishing the favorable driving force for Mg^{2+} influx via the TRPM6 channels.

Autosomal Dominant Hypomagnesemia

$K_v1.1$ is a Shaker-related voltage-gated K^+ channel encoded by *KCNA1*. Its mutation, c.763A>G (p.Asn255Asp), has been shown to cause autosomal dominant hypomagnesemia [73]. It is posited that the substitution of the highly conserved asparagine for aspartic acid renders the $K_v1.1$ channel nonfunctional. The mutation was identified in a large Brazilian family. Of the 46 family members, 21 carried the mutation and were affected by severe hypomagnesemia. Affected individuals experience muscle cramps, tetanic episodes, muscle weakness, and myokymia [73]. Remarkably, $K_v1.1$ mutations have been identified previously and have been associated with episodic ataxia without Mg^{2+} alteration [95]. The previously described mutations are adjacent to the mutations that are responsible for hypomagnesemia. It is hypothesized that replacement of a neutral amino acid (asparagine) with an acidic (aspartic) acid – which alters the structure of $K_v1.1$, resulting in a defect in apical K^+ efflux via $K_v1.1$ – compromises the generation of the transapical membrane potential, leading to a defect in Mg^{2+} absorption.

Isolated Dominant Hypomagnesemia

To date, a total of 3 families with hypomagnesemia related to an *FXYD2* mutation have been identified. These 3 families have the identical c.115G>A (p.Gly41Arg) mutation. The first family reported with this mutation and hypomagnesemia was a Dutch family [56, 96, 97]. Recently, 2 additional families were identified, from Belgium and the Netherlands [98]. The affected individuals show hypomagnesemia and hypocalcemia. Some of them have polyuria and hypokalemia. All of the affected individuals complained of muscle cramps and generalized weakness. Kidney failure was reported in one of the probands. Interestingly, haplotype analyses suggest that the 3 families have a common founder, but genealogy failed to identify a common ancestor up to 1700 [98].

Hypomagnesemia and Maturity-Onset Diabetes of the Young

Patients with dominant mutations in the *HNF1B* gene or recessive mutations in the *PCBD1* gene can develop hypomagnesemia and maturity-onset diabetes of the young [58, 99]. These clinical findings could be explained at the molecular level by the role of PCBD1 as a transcriptional coactivator of HNF1B. *HNF1B* mutations have also been associated with polycystic kidney disease and urogenital malformations, consistent with the role of HNF1B in transcriptional activation of *PKHD1* [61] and organogenesis [59, 60, 62]. Not all patients harboring these mutations, however, develop hypomagnesemia [58, 100]. Further studies are necessary to better understand the underlying genotype-phonotype relation.

Dominant Hypomagnesemia due to CNNM2 Mutations

Two unrelated families with dominant hypomagnesemia were found to carry two mutations in the *CNNM2* gene [78, 101]. One is the heterozygous deletion c.117delG (p.Ile40SerfsX15), and the other is the heterozygous missense mutation c.1703C>T (p.Thr568Ile). The deletion causes truncated proteins and the missense mutation causes a substitution of an amino acid for one of the two highly conserved CBS domains. Significant intrafamilial phenotypic variation was observed. In the family with the truncating mutation, the proband developed symptomatic hypomagnesemia at the age of 2 years, while his mother developed symptoms in her teenage years.

Remaining Questions and Future Research

Although our understanding of Mg^{2+} regulation, especially in the kidneys, has improved tremendously in the space of the last 20 years, a large number of questions remain. For instance, most of the Mg^{2+} transporter-mediated signaling pathways have not been fully elucidated; the underlying reasons for the large and complex variations in genotype and phenotype are unknown, as are the epigenetic factors and genetic modifiers that may influence the clinical phenotypes. Moreover, whether the other TAL-expressed claudins (i.e., claudin-10) perform a role in Mg^{2+} homeostasis is unclear. Mg^{2+} dysregulation in mitochondrial diseases has not been well characterized. Lastly, many proposed disease mechanisms are speculative (i.e., the pathophysiology of renal failure in FHHNC). That said, with emerging modern technologies – i.e., high-resolution microscopy and crystallography, which has resolved the crystal structures of claudin [102] and Mg^{2+} transporter proteins (Mrs2 and SLC41A) [79, 103–105], newer electrophysiology techniques, genome-wide association studies, next-generation sequencing technology, and analytical tools – it is foreseeable that, before long, more information on Mg^{2+} regulation and dysregulation will be revealed. Studies on genetic alterations would help us to manage the common clinical patients with dysmagnesemia.

Conclusion

Mg^{2+} is a critical cation, inextricably intertwined with numerous cellular functions to maintain cell viability and tissue integrity. Dysmagnesemia and polymorphisms of genes related to Mg^{2+} regulation have been associated with a number of diseases including cancer, diabetes, nephrolithiasis, osteoporosis, and an array of neurological abnormalities. The genetics of Mg^{2+} dysregulation are heterogeneous and complex. Although significant progress has been made, the quest for more details and for the remaining unknowns continues. These efforts are well justified, since understanding the underlying regulations and abnormalities will facilitate a better diagnosis, prevention, and management of nongenetic forms of dysmagnesemia which are common and can be associated with devastating consequences [2].

Conflict of Interest Statement

The authors have no competing interests.

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