Role of the distal convoluted tubule in renal Mg²⁺ handling: molecular lessons from inherited hypomagnesemia

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Abstract. In healthy individuals, Mg²⁺ homeostasis is tightly regulated by the concerted action of intestinal absorption, exchange with bone, and renal excretion. The kidney, more precisely the distal convoluted tubule (DCT), is the final determinant of plasma Mg²⁺ concentrations. Positional cloning strategies in families with hereditary hypomagnesemia identified defects in several proteins localized in the DCT as causative factors. So far, the identified actors involved in Mg²⁺ handling in the DCT include: the transient receptor potential channel melastatin member 6, the pro-epidermal growth factor, the thiazide-sensitive Na⁺-Cl⁻ cotransporter, the γ-subunit of the Na⁺/K⁺-ATPase, the hepatocyte nuclear factor 1B, the potassium channels Kv1.1 and Kir4.1, and the cyclin M2. In the years to come, the identification of new magnesiotropic genes and related proteins will further clarify the role of the kidney in Mg²⁺ homeostasis, and will potentially lead to new therapeutic approaches for hypomagnesemia.

Key words: familial hypomagnesemia, distal convoluted tubule, transcellular transport

In the human body, Mg²⁺ homeostasis relies on tissues transporting and/or storing Mg²⁺ (mainly kidney, intestine and bone), hormones that modulate its mobilization [1], and sensors controlling the release of magnesotropic hormones and regulation of the transport of Mg²⁺ in tissues [2, 3]. The dynamic actions of these homeostatic elements guarantee the maintenance of normal plasma Mg²⁺ concentrations within a narrow range (0.7-1.1 mmol/L). In particular, the kidney is the final determinant of circulating Mg²⁺ levels. Under physiological conditions, the kidney filters 80% of the total plasma Mg²⁺, but the tight regulation of the reabsorption processes that take place along the nephron guarantee less than 5% Mg²⁺ waste in the urine. In short, 10 to 20% of the filtered Mg²⁺ is reabsorbed by the proximal tubule (PT), whereas the majority (65-70%) is reabsorbed in the cortical thick ascending limb (TAL) of Henle’s loop. Here, the positive transepithelial voltage drives Mg²⁺ across a paracellular pathway that involves the tight junction proteins, claudin-16 and claudin-19 [4, 5]. Defects in either protein are causative for familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC; OMIM 248250); transgenic mice lacking either claudin-16 or claudin-19 reproduce many of the features of FHHNC [5]. Of the filtered Mg²⁺, 10-15% reaches the distal convoluted tubule (DCT), where 70 to 80% is reabsorbed. In the DCT, the transepithelial voltage is negative and Mg²⁺ reabsorption occurs in an active transcellular manner. As the more distal nephron segments...
are largely impermeable to Mg²⁺, the DCT plays an important role in determining the final urinary excretion [6].

The DCT consists of two subsegments named DCT1 and DCT2, with DCT1 being primarily involved in overall Mg²⁺ handling. In the DCT1, Mg²⁺ transport from the pro-urine into the cell is mediated by the apical transient receptor potential melastatin subtype 6 (TRPM6), and is critically influenced by ATP-dependent Na⁺ reabsorption [7, 8] and cellular energy metabolism [9].

So far, the basolateral mechanism responsible for Mg²⁺ transport from the intracellular compartment towards the blood remains unknown.

Hypermagnesemia (serum Mg²⁺ > 1.1 mmol/L) is a rare condition and mainly occurs in cases of renal insufficiency. Dialysis represents the most rapid therapeutic intervention to lower Mg²⁺ levels. On the other hand, hypomagnesemia (serum Mg²⁺ < 0.70 mmol/L) is a common electrolyte disturbance observed in up to 12% of hospitalized patients and 65% of patients in the Intensive Care Unit. The clinical manifestations in patients with hypomagnesemia include neuromuscular irritability, such as tetany and seizures, cardiac arrhythmias, secondary hypocalcemia and secondary hypokalemia. Patients suffering from severe hypomagnesemia are often supplemented with Mg²⁺. Acute intravenous infusion is usually reserved for patients with symptomatic hypomagnesemia, whereas in asymptomatic hypomagnesemic patients, oral replacement represents the preferred route of administration, even if limited by the amount of Mg²⁺ that causes diarrhea.

Hypomagnesemia can be primary to homeostatic defects, i.e. either intestinal or renal losses. Measurement of urinary Mg²⁺ excretion before Mg²⁺ replacement helps to determine whether the hypomagnesmic state results from renal or extra-renal causes. Moreover, hypomagnesemia can be secondary to treatment with therapeutic agents [10-12] or to other medical conditions [13]. In recent years, several, rare, inherited forms of hypomagnesemia have been described in the literature [14]. Inherited hypomagnesemia manifests mostly during infancy when serum Mg²⁺ levels can vary from slightly below the physiological concentration to up to <0.20 mmol/L. Genome-wide linkage analysis for affected family members have allowed the identification of new genes and derived proteins involved in renal Mg²⁺ handling (figure 1).

This review aims to summarize genetic and functional findings that elucidated the pathophysiology underlying inherited forms of hypomagnesemia that affect Mg²⁺ transport in the DCT.

**Figure 1.** Schematic representation of the molecular players in which defects cause dysfunctional Mg²⁺ handling in the distal convoluted tubule (DCT).
Monogenic defects disrupting Mg\textsuperscript{2+} reabsorption in the DCT

**Transient receptor potential channel melastatin member 6**

Two independent research groups identified the transient receptor potential channel melastatin member 6 (TRPM6) as the causative gene in the autosomal recessive disorder called hypomagnesemia with secondary hypocalcemia (HSH, OMIM 602014) [15, 16]. HSH patients develop severe hypomagnesemia (0.1-0.4 mmol/L), secondary hypocalcemia, disturbed neuromuscular excitability, muscle spasms, tetany and convulsions. Using immunohistochemistry, the TRPM6 protein was shown to localize at the apical membrane of the DCT1 and at the brush-border membrane of the intestine [17]. Indeed, the homozygous and compound heterozygous mutations identified in HSH patients lead to both impaired intestinal Mg\textsuperscript{2+} absorption and renal re-absorption [15, 16]. TRPM6, and its closest homologue TRPM7, are unique epithelial Mg\textsuperscript{2+} channels that couple ion transport to a \( \theta \)-kinase activity. In recent years, intense research has focused on the regulatory factors of the TRPM6 \( \alpha \)-kinase domain [18]. Overall, TRPM6 can be defined as the gatekeeper of Mg\textsuperscript{2+} homeostasis, and many proteins that are found mutated in inherited forms of hypomagnesemia directly affect TRPM6 or alter the driving force for Mg\textsuperscript{2+} influx via the channel.

**Epidermal growth factor**

Using a whole-genome linkage analysis, mutation c.3209C>T (p.Pro1070Leu) in the EGF gene was shown to be the causative defect in recessive isolated renal hypomagnesemia (IRH, OMIM 611718) [19]. IRH was originally described by Geven et al. in two siblings who presented epileptic seizures, moderate mental retardation, hypomagnesemia (0.53-0.66 mmol/L) and normal Ca\textsuperscript{2+} handling [20]. The EGF gene encodes for pro-EGF, a small peptide hormone expressed in several organs, including gastrointestinal tract, respiratory tract, and kidney (primarily DCT). Here, after insertion at the basolateral membrane, pro-EGF is processed into soluble EGF that is able to stimulate locally the EGF receptor (figure 1). EGF was defined as the first magnesiotropic hormone when patch clamp analysis revealed that EGF significantly stimulated the activity of TRPM6. Later, biochemical experiments reported that EGF increases TRPM6 cell surface abundance by redistributing the channel from intracellular vesicles to the plasma membrane via signaling through Src kinases and Rac1 [21]. The mutation observed in the family with IRH, disrupts the basolateral-sorting motif in pro-EGF. Thus, mutated pro-EGF is impaired in its routing towards the basolateral membrane, leading to disturbed stimulation of the EGFR and subsequent reduced Mg\textsuperscript{2+} transport by TRPM6.

**NaCl cotransporter**

Gitelman syndrome (GS, OMIM 263800) is an autosomal recessive disorder characterized by hypokalemic metabolic alkalosis, hypomagnesemia, hypocalciuria and commonly observed periods of muscle weakness and tetany. The underlying causes for GS are homozygous or compound heterozygous mutations or deletions in the SLC12A3 gene encoding for the thiazide-sensitive NaCl cotransporter (NCC) [7]. NCC is expressed at the apical membrane of the DCT1, where it facilitates reabsorption of Na\textsuperscript{+} and Cl\textsuperscript{-} from the pro-urine into the cell (figure 1). Inactivating mutations in NCC, by chronic thiazide administration, causes renal NaCl wasting. This in turn leads to extracellular volume contraction with subsequent activation of the renin-angiotensin-aldosterone system, increased Na\textsuperscript{+} reabsorption in the more distal segments of the nephron in exchange for K\textsuperscript{+}, and thereby development of hypokalemic alkalosis. The disturbances in mineral metabolism observed in the course of GS are secondary to the defects in NCC. The observed hypocalciuria is caused by an increased proximal tubular reabsorption in response to the mild volume depletion. The exact mechanism at the basis of the hypomagnesemia remains to be determined, but is likely due to a reduced abundance of TRPM6, leading to renal Mg\textsuperscript{2+} wasting. It has been suggested that increased aldosterone levels may be the reason for the decreased TRPM6 expression [22].

**\( \gamma \)-Subunit of the Na\textsuperscript{+}/K\textsuperscript{+}-ATPase**

Isolated dominant hypomagnesemia with hypocalciuria (IDH, OMIM 154020) is caused by a
c.121G>A (p.Gly41Arg) mutation in the FXYD2 gene, encoding for the γ-subunit of the Na+/K+-ATPase [23]. Patients with IDH also suffered from convulsions, probably due to the low serum Mg²⁺ levels (<0.4mmol/L). The γ-subunit (FXYD2) is a type I transmembrane protein that modulates the kinetics of the Na+/K+-ATPase by altering its affinity for K⁺ and Na⁺ at the basolateral membrane of kidney tubules [24]. The Na⁺/K⁺-ATPase, in turn, guarantees a local negative membrane potential that drives the trans-epithelial salt reabsorption. Upon oligomerization, the mutated γ-subunit impairs routing of the wild type protein towards the plasma membrane, in accordance with the dominant inheritance of the disease [25]. Of note, the FXYD2 gene can be alternatively transcribed into two main variants, namely γa and γb, that differ only at their extracellular N-termini [26]. Immunostaining of γb has been found specifically in DCT1, whereas γa is more broadly expressed along the nephron [27]. The p.Gly41Arg mutation localizes in the unique transmembrane domain of the protein and therefore, affects both isoforms. However, the exact molecular mechanism by which the γ-subunit ultimately modulates Mg²⁺ handling in DCT remains to be established. Speculatively, mutation of the γ-subunit causes a reduction in the activity of the Na⁺/K⁺-ATPase that induces depolarization of the apical membrane and decreases the Mg²⁺ transport via TRPM6. Alternatively, the γ-subunit may work as an accessory protein of other Mg²⁺-related protein complexes.

**Hepatocyte nuclear factor 1 homeobox B**

Heterozygous mutations in the HNF1B gene are responsible for a dominant renal cysts and diabetes syndrome (RCAD, OMIM 137920) that includes both renal and extrarenal manifestations. HNF1B encodes for the transcription factor hepatocyte nuclear factor 1 homeobox B that is critically involved in early vertebrate development and tissue-specific transcription in kidney, liver, pancreas and genital tracts [28, 29]. Defects in the HNF1B gene include whole-gene deletion in about 50% of the patients, whereas point mutations are detected in most of the remaining cases, along the entire coding sequence. In a large cohort of patients, no correlation was found between the type of mutation and the type and/or severity of renal disease [30]. Renal Mg²⁺ wasting with hypocaliuria occurs in up to 50% of the cases. Moreover, HNF1B nephropathy in adulthood is accompanied by hypokalemia in nearly half of the cases [31]. So far, screening for HNF1B binding sites in genes known to affect renal Mg²⁺ transport has revealed a role for HNF1B in the transcriptional regulation of the FXYD2 gene [32], and in particular of the promoter leading to the γ-subunit protein [27]. Nevertheless, the molecular mechanisms responsible for the hypomagnesemic phenotype remain unknown. In the future, a comprehensive study of serum Mg²⁺ values in HNF1B families, and a genotype-phenotype correlation between the molecular basis of Mg²⁺ wasting in the DCT.

**Kv1.1**

A positional cloning approach in a family with isolated autosomal dominant hypomagnesemia lead to the identification of a c.763A>G (p.Asns55Asp) mutation in the KCNA1 gene, encoding the voltage-gated potassium (K⁺) channels Kv1.1 [33]. The affected family members present with recurrent muscle cramps, tetany, tremor, muscle weakness, cerebellar atrophy, and myokymia, in addition to low serum Mg²⁺ levels (0.28-0.37 mmol/L). When overexpressed in human embryonic kidney cells, Kv1.1 hyperpolarizes the plasma membrane compared to mock DNA transfected cells. The conserved asparagine at position 255 localizes in the third transmembrane segment of Kv1.1 and was found to be necessary for the voltage dependence and kinetics of channel gating [34]. Substitution of the asparagine for aspartic acid leads to a non-functional channel, with a dominant negative effect on the wild type Kv1.1 [33]. Localization of Kv1.1 in DCT1 suggests that Kv1.1 might establish a favorable luminal membrane potential in DCT cells to control TRPM6-mediated Mg²⁺ reabsorption.

Previously identified mutations in Kv1.1 are characterized by episodic ataxia (EA1, OMIM 160120) [35, 36], but show no hypomagnesemia. On the other hand, family members with the p.Asns55Asp mutation showed slight atrophy of the cerebral vermis and myokymic discharge [33]. Further studies should elucidate how distinct mutations in Kv1.1 can result in the tissue-specific phenotypes.
Kir4.1

Mutations in the KCNJ10 gene, encoding an inwardly rectifying K⁺ channel (Kir4.1), were identified as causative defects for a new syndrome of hypomagnesemia. Affected patients suffered from seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance (SeSAME) [37]/epilepsy, ataxia, sensorineural deafness, and renal tubulopathy (EAST) [38] syndrome (OMIM 612780). The renal phenotype associated with loss of KCNJ10 function comprises a state of Gitelman-like salt wasting, with hypokalemic metabolic alkalosis, hypomagnesemia and hypocalcemia. Positive Kir4.1 immunostaining was found at the basolateral membrane of the DCT. Here, Kir4.1 allows K⁺ recycling to enable normal activity of the Na⁺/K⁺-ATPase (figure 1). The Na⁺/K⁺-ATPase generates a negative membrane potential and provides a Na⁺ gradient for NCC to facilitate transport of Na⁺ from the lumen into the cytoplasm. Impairment of the electrogenic Na⁺/K⁺-ATPase activity at the basolateral membrane due to Kir4.1 loss of function, may result in depolarization of the apical membrane and therefore reduction of the Mg²⁺ influx via TRPM6. It was recently shown that CaSR interacts with and inactivates Kir4.1 [39]. Evidence for this interaction suggests that hypomagnesemia occurring in patients with activating mutations in CaSR [40] might affect renal Na⁺ transport, and therefore causes Mg²⁺ wasting, via the same mechanisms as loss of function mutations in Kir4.1. Kir4.1 is often found as a heteromeric complex with Kir5.1. Interestingly, in Kir5.1 knock-out mice (Kir5.1⁻/⁻), homomeric Kir4.1 channels are pH₇-insensitive and constitutively highly active. This increases the basolateral K⁺ conductance in the DCT leading to the development of a mirror-image phenotype to SeSAME/EAST syndrome [41].

CNNM2

Among the numerous proteins associated with Mg²⁺ transport in eukaryotes [42], cyclin M2 (CNNM2) certainly plays a role in Mg²⁺ homeostasis. Early findings suggested the involvement of CNNM2 in Mg²⁺ handling, based on a microarray and a genome-wide association study [43, 44]. Using a candidate screening approach in two unrelated families with unexplained dominant hypomagnesemia (serum Mg²⁺ 0.51-0.36 mmol/L, OMIM 613882), Stuiver et al. identified two mutations in the CNNM2 gene: the heterozygous deletion c.117delG and the heterozygous missense mutation c.1703C>T [45]. The deletion c.117delG (p.Ile40SerfsX15) leads to a truncated protein with uncharacterized function, whereas the missense mutation c.1703C>T (p.Thr568Ile) causes a substitution in one of the two highly conserved cystathionine beta-synthase (CBS) domains, known to be important for dimerization of several transporters as well as for Mg²⁺-dependent gating of bacterial MgtE channel [46]. Patch clamp experiments with wild type CNNM2 in human embryonic kidney cells revealed a Mg²⁺-sensitive Na⁺ current that was clearly diminished in p.Thr568Ile mutant CNNM2 transfected cells [45]. Three splice variants of CNNM2 have been identified in mammals. The p.Thr568Ile substitution affects the coding sequence of variant 1 and 2 of CNNM2 [47]. Although only variant 1 seems to be involved in Mg²⁺ transport [47], in the future, the physiological relevance of the two isoforms and their effect on the wild type protein should be investigated. Interestingly, CNNM2 shows a clear, basolateral localization, both in the thick ascending limb of Henle’s loop and the DCT of the kidney, appointing CNNM2 as a putative candidate for an Mg²⁺ extrusion mechanism. Nevertheless, the wide expression pattern of CNNM2 being present in many tissues, and no evidence of a CNNM2-mediated Mg²⁺ current in mammalian cells point more likely to a Mg²⁺-sensing function of the protein rather than its direct role in transcellular Mg²⁺ transport in the DCT [45].

Conclusion

In summary, genetic studies in cases of familial hypomagnesemia revealed defects in several proteins, with a predominant expression in the DCT1 of the kidney (figure 1). Elucidation of the molecular origin underlying these defects has greatly increased our understanding of renal Mg²⁺ handling in physiological conditions. Further studies are needed to investigate thoroughly the hormonal regulation, the interacting partners, the intracellular signaling pathways and the post-transcriptional events that modulate the activity of the currently known magnesiotropic proteins. In the future, the successful collaboration between
clinical geneticists and renal physiologists will allow the identification of new disease genes and, therefore, lead to the functional characterization of additional molecular players involved in active, renal Mg\textsuperscript{2+} transport.

**Disclosure**

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