New molecular players facilitating Mg^{2+} reabsorption in the distal convoluted tubule

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The renal distal convoluted tubule (DCT) has an essential role in maintaining systemic magnesium (Mg^{2+}) concentration. The DCT is the final determinant of plasma Mg^{2+} levels, as the more distal nephron segments are largely impermeable to Mg^{2+}. In the past decade, positional candidate strategies in families with inherited forms of hypomagnesemia have led to the identification of genes involved in Mg^{2+} handling. A large fraction of this resides in the DCT, namely, (i) the transient receptor potential channel melastatin subtype 6 (TRPM6), a divalent cation-permeable channel located at the luminal membrane of the DCT, facilitates Mg^{2+} entry from the pro-urine into the cell; (ii) the epidermal growth factor is a novel hormone regulating active Mg^{2+} transport through TRPM6; (iii) the voltage-gated K^+ channel, Kv1.1, establishes a favorable luminal membrane potential for TRPM6-mediated Mg^{2+} transport; (iv) the Na^+/K^+-ATPase γ-subunit (γ-Na^+/K^+-ATPase) was identified as mutated protein in a family with isolated dominant hypomagnesemia. The molecular mechanism by which γ-Na^+/K^+-ATPase is involved in DCT Mg^{2+} handling remains unknown; (v) a high percentage of patients with mutations in the renal transcription factor *HNF1B* (hepatocyte nuclear factor 1 homeobox B) gene develop hypomagnesemia; and (vi) Gitelman and EAST/SeSAME syndrome patients suffer from a similar tubulopathy due to mutations in NCC (NaCl cotransporter) and Kir4.1, respectively. In these patients, decreased expression of TRPM6 is proposed to cause hypomagnesemia. Insights into the molecular mechanisms of the identified genes, as well as the identification of novel genes, will further improve our knowledge about renal Mg^{2+} handling.

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Magnesium (Mg^{2+}) is a versatile electrolyte shown to be involved in many cellular processes. It functions as a cofactor in the energy metabolism, nucleotide and protein synthesis, and as a regulator of sodium (Na^+), potassium (K^+), and calcium (Ca^{2+}) channels. To maintain these cellular functions, plasma Mg^{2+} levels have to be kept within a narrow range (0.70–1.1 mmol/l). A representative study showed that a surprisingly high percentage of hospitalized patients (acute 26.1% and chronic 3.5%) are diagnosed with hypomagnesemia.[1] Hypomagnesemia is observed under various conditions (i) by use of drugs such as the immunosuppressive agent, cyclosporine,[2] anti-acidic drugs like omeprazole and esomeprazole,[3,4] and anticancer drugs like cetuximab[5,6] and cisplatin;[7] (ii) by inherited forms; and (iii) secondary to other medical conditions like diabetes mellitus type II. Symptoms of hypomagnesemia include muscle cramps, tremors, tetany, a short QT interval on the electrocardiography, and in some instances, cardiac arrhythmia. Persistent hypomagnesemia can eventually cause death. Patients suffering from severe hypomagnesemia are often supplemented with Mg^{2+}. A high dose of Mg^{2+}, however, can have serious adverse effects such as diarrhea and abdominal cramping. Furthermore, magnesium salts are often given in case of severe asthma attacks[8] and to treat pre-eclampsia in pregnant women.[9] The molecular mechanism by which Mg^{2+} improves the pathological conditions is at this point unknown.

Three organs determine the plasma Mg^{2+} level, namely, the intestine by which Mg^{2+} is taken up from the food, bones, which store and release Mg^{2+}, and the kidney, which determines the excretion of Mg^{2+}. The intake of Mg^{2+} is ~300–350 mg/day of which 40–60% is absorbed by the intestine.[10] Mg^{2+} absorption takes place along the intestinal tract by passive para- or active transcellular pathways.[11] With normal dietary content, Mg^{2+} is most efficiently absorbed in the distal part of the small bowel in a passive manner. When Mg^{2+} intake is low, the Mg^{2+} absorption is increased through active transport systems in the large intestines.[11,12] The highest percentage (50–60%) of total body Mg^{2+} is stored in the skeleton. It is hypothesized that bone serves as a buffer for plasma Mg^{2+}. At this point, little is known about the mechanisms by which Mg^{2+} is stored in bone by osteoblasts and released by osteoclasts.[13,14] The kidneys are

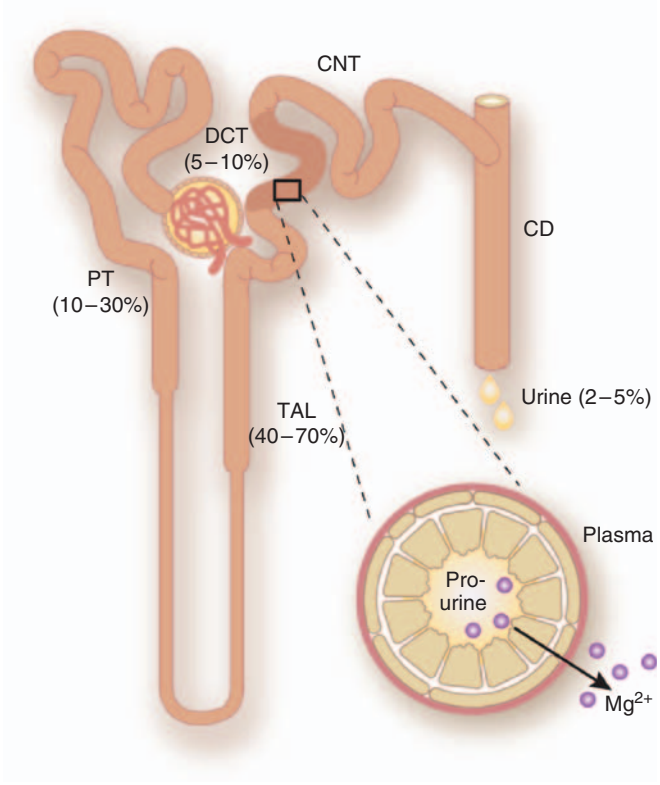

Figure 1 | Renal Mg^{2+} reabsorption. The glomerulus filters Mg^{2+}, of which 90–95% is subsequently reabsorbed along the nephron. Approximately 10–30% of the Mg^{2+} is reabsorbed by the proximal tubule in a passive manner. The highest level is reabsorbed by the thick ascending loop of Henle (TAL) (40–70%). In this part of the nephron, Mg^{2+} transport is facilitated in a passive paracellular manner by tight junction proteins claudin-16 and claudin-19. Only 5–10% of the filtered load is reabsorbed in the distal convoluted tubule (DCT); however, this segment determines the final Mg^{2+} concentration through active transcellular transport. CD, collecting duct; CNT, connecting tubule; PT, proximal tubule.

involved in the regulation and fine-tuning of the final Mg^{2+} concentration in plasma. Each day, ~2500 mg of Mg^{2+} is filtered by the glomeruli of which 90–95% is reabsorbed along the nephrons (Figure 1). The highest level of reabsorption occurs in the proximal tubules and the thick ascending limbs of Henle's loop (TAL) in a passive paracellular manner (10–30% and 40–70%, respectively) (Figure 1). The mechanisms that manage Mg^{2+} transport in the proximal tubules are unknown, whereas in the TAL, Mg^{2+} reabsorption is facilitated by the tight junction proteins, claudin-16[15] and claudin-19[16] (Figure 1). Mutations in claudin-16 and claudin-19 are causative for familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC; OMIM 248250). A recent study showed that the interaction between tight junction proteins, claudin-16 and claudin-19, forms a specific cation-permeable channel. FHHNC mutations have been shown to disrupt the cation-selective properties of the claudin-16 and claudin-19 channels. This presumably disrupts the lumen positive potential that generates passive paracellular transport of Mg^{2+}.[17] The final 5–10% of the filtered load is reabsorbed by the distal convoluted tubule (DCT) (Figure 1), which consists of two subsegments, namely, DCT1 and DCT2. The DCT1 segment determines the final Mg^{2+} concentration, as the more distal parts of the tubule are largely impermeable to Mg^{2+}. In DCT1, Mg^{2+} reabsorption occurs in an active transcellular manner through previously unknown mechanisms (Figure 1). In recent years, positional candidate approaches in families with monogenetic forms of hypomagnesemia have allowed the identification of new genes and derived proteins involved in active renal Mg^{2+} handling. This review provides an overview of the most recent findings.

PATHOPHYSIOLOGY OF MONOGENETIC DISORDERS IN HYPOMAGNESEMIA

Transient receptor potential channel melastatin member 6

Walder *et al.* reported three consanguineous kindreds suffering from hypomagnesemia and secondary hypocalcemia (HSH; OMIM 602014; Table 1). The phenotype manifested 2–8 weeks after birth and consisted of neurological symptoms such as tetany, muscle spasms, and seizures. These patients display low plasma Mg^{2+} levels (0.1–0.4 mmol/l) that are caused by defective intestinal and renal absorption of Mg^{2+}.[18] The low plasma Ca^{2+} levels are secondary, likely due to parathyroid failure caused by hypomagnesemia (Table 1). Hypomagnesemia blocks the secretion of parathyroid, hence resulting in decreased reabsorption of Ca^{2+} by the kidney.[19] A whole-genome scanning approach showed linkage to chromosome 9p22.[18] In the following years, two groups independently identified new HSH families that were used to narrow down the critical region by use of haplotyping analysis. Subsequent screening for candidate genes in the mapped region resulted in the identification of homozygous and compound heterozygous mutations in the transient receptor potential channel melastatin member 6 (*TRPM6*; OMIM 607009) gene (Table 1).[20,21] By use of immunohistochemistry, the TRPM6 protein was shown to localize to the luminal membrane of DCT cells and the brush-border membrane of the intestine (Figure 1).[22] The closest relative of TRPM6 is TRPM7, which is ubiquitously expressed. A striking feature of both channels is the α-kinase domain, which is located at the intracellular carboxy (C)-terminus. Functional analysis identified TRPM6 as a Mg^{2+}- and Ca^{2+}-permeable channel, although the affinity for the latter ion is five times lower (Figure 2).[22] The α-kinase domain is proposed to function as a sensor of the intracellular Mg^{2+} concentration. As a consequence, the Mg^{2+} influx through TRPM6 is regulated, preventing intracellular Mg^{2+} overload. Recently, a receptor for activated C-kinase 1 and repressor of estrogen receptor activity were identified as the TRPM6 α-kinase domain-interacting proteins. Receptor for activated C-kinase 1 and repressor of estrogen receptor activity were shown to function as a dynamic switch controlling TRPM6 channel activity through the α-kinase domain. Moreover, TRPM6 is inhibited on