

## Regulation of magnesium balance: lessons learned from human genetic disease

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### Abstract

Magnesium ( $Mg^{2+}$ ) is the fourth most abundant cation in the body. Thus, magnesium homeostasis needs to be tightly regulated, and this is facilitated by intestinal absorption and renal excretion. Magnesium absorption is dependent on two concomitant pathways found in both in the intestine and the kidneys: passive paracellular transport via claudins facilitates bulk magnesium absorption, whereas active transcellular pathways mediate the fine-tuning of magnesium absorption. The identification of genes responsible for diseases associated with hypomagnesaemia resulted in the discovery of several magnesiotropic proteins. Claudins 16 and 19 form the tight junction pore necessary for mass magnesium transport. However, most of the causes of genetic hypomagnesaemia can be tracked down to transcellular magnesium transport in the distal convoluted tubule. Within the distal convoluted tubule, magnesium reabsorption is a tightly regulated process that determines the final urine magnesium concentration. Therefore, insufficient magnesium transport in the distal convoluted tubule owing to mutated magnesiotropic proteins inevitably leads to magnesium loss, which cannot be compensated for in downstream tubule segments. Better understanding of the molecular mechanism regulating magnesium reabsorption will give new opportunities for better therapies, perhaps including therapies for patients with chronic renal failure.

**Keywords:** human genetic disease; hypomagnesaemia; magnesium homeostasis; TRPM6

### Introduction

As magnesium ( $Mg^{2+}$ ) is a cofactor of many enzymes, it is involved in all major cellular processes such as energy metabolism, DNA transcription and protein synthesis. Physiologically,  $Mg^{2+}$  plays an essential role in bone formation, neuromuscular stability and muscle contraction. Therefore, the tight regulation of plasma  $Mg^{2+}$  levels is of vital importance. Hypomagnesaemia occurs because of decreased gastrointestinal absorption or increased renal  $Mg^{2+}$  excretion and is associated with a wide spectrum of diseases, including Type 2 diabetes, hypertension, osteoporosis, tetany, seizures and depression [1–4]. This issue has been discussed in greater detail in the review by Jahnen-Dechent and Ketteler [5] and Geiger and Wanner [6] in this supplement. Certain drug therapies (e.g. diuretics, aminoglycosides, cetuximab therapy and immunosuppressive agents) can result in acquired renal  $Mg^{2+}$  wasting and associated low serum  $Mg^{2+}$  levels [7]. Hypomagnesaemia can be treated with oral  $Mg^{2+}$  supplements, though at high doses these might cause diarrhoea. Oral  $Mg^{2+}$  supplements are also often given with potassium ( $K^+$ ) supplements because  $Mg^{2+}$  deficiency is frequently associated with hypokalaemia [8]. In severely hypomagnesaemic patients, intravenous supplementation can be essential to restore the patient's  $Mg^{2+}$  levels without discomforting the patient. During the last decade, human hereditary disorders have given great insight into the molecular pathways involved in the regulation of

$Mg^{2+}$  homeostasis (Table 1). In this review, we will focus on the regulation of magnesium homeostasis and we will describe the genetic causes of hypomagnesaemia and the underlying molecular mechanisms.

### Regulation of magnesium homeostasis

Many studies have shown intestinal  $Mg^{2+}$  absorption is balanced against renal  $Mg^{2+}$  excretion [9–11]. In times of a temporary  $Mg^{2+}$  deficit, the body depends on the availability of  $Mg^{2+}$  in bone to maintain constant serum levels [12]. Therefore,  $Mg^{2+}$  homeostasis depends on three organs: the intestine, facilitating  $Mg^{2+}$  uptake; bone, the  $Mg^{2+}$  storage system of the body and the kidneys, which are responsible for  $Mg^{2+}$  excretion.

#### Intestinal magnesium uptake

In healthy people,  $Mg^{2+}$  blood plasma concentrations range between 0.65 and 1.05 mmol/L. In order to maintain these levels, a daily  $Mg^{2+}$  intake of 320 mg for men and 420 mg for women is recommended by the US Food and Nutrition Board. Approximately 30–50% of dietary  $Mg^{2+}$  is absorbed by the intestine (Figure 1). However, when  $Mg^{2+}$  intake is low, the absorption percentage can rise to ~80% [13].  $Mg^{2+}$  absorption takes place mainly in the distal small intestine and in the colon [14]. Thus, shortening of

**Table 1.** Human genetic magnesium transport disorders<sup>a</sup>

Disease	OMIM	Renal segment	Gene	Protein	Protein full name	Serum Mg <sup>2+</sup>	Urine Mg <sup>2+</sup>	Other symptoms
Familial hypomagnesaemia with hypercalciuria and nephrocalcinosis	248250	TAL	<i>Claudin-16</i> and <i>Claudin-19</i>	Claudin-16 and claudin-19	Claudin-16 and claudin-19	↓	↑	Nephrocalcinosis and visual impairment
Bartter's syndrome	241200	TAL	<i>SLC12A1</i> , <i>BSND</i> , <i>CLCNKB</i> , <i>KCNJ1</i>	NKCC, Barttin, ClC-Kb, ROMK	Na <sup>+</sup> -K <sup>+</sup> -2Cl <sup>-</sup> cotransporter, Barttin, ClC-Kb Cl channel, ROMK K channel	↓	↓	Hypokalaemic alkalosis, elevated renin and aldosterone
Hypomagnesaemia with secondary hypocalcemia	602014	DCT	<i>TRPM6</i>	TRPM6	Transient receptor potential melastatin member 6	↓	↑	Epileptic seizures, muscle spasms and mental retardation
Isolated autosomal-recessive hypomagnesaemia	611718	DCT	<i>EGF</i>	EGF	Epidermal growth factor	↓	↑	Epileptic seizures and mental retardation
Autosomal-dominant hypomagnesaemia	176260	DCT	<i>KCNA1</i>	Kv1.1	Voltage-gated K channel 1.1	↓	↓	Muscle cramps, tetany and tremor
Gitelman syndrome	263800	DCT	<i>NCC</i>	NCC	Na-Cl cotransporter	↓	↑	Muscle weakness, tetany and fatigue
Isolated dominant hypomagnesaemia	154020	DCT	<i>FXD2</i>	Na <sup>+</sup> /K <sup>+</sup> -ATPase	Na <sup>+</sup> /K <sup>+</sup> -ATPase	↓	↑	Convulsions
Maturity-onset diabetes of the young	137920	DCT	<i>HNF1B</i>	HNF1B	Hepatocyte nuclear factor 1	↓	↑	Neonatal diabetes and renal malformation
SeSAME syndrome	612780	DCT	<i>KCNJ10</i>	Kir4.1	Kir4.1 K channel	↓	↓	Sensorineural deafness, seizures and mental retardation

<sup>a</sup>Overview of genetic disease associated with hypomagnesaemia, the genes held responsible. The table shows which proteins are mutated and the renal segment in which they are expressed. Effects on serum and urine Mg<sup>2+</sup> concentrations and other symptoms of the disease are shown in the last columns. TAL, thick ascending limb of Henle's loop; DCT, distal convoluted tubule; OMIM, online Mendelian inheritance in man.

the rat ileum, for example, results in a substantial decrease of Mg<sup>2+</sup> absorption [15].

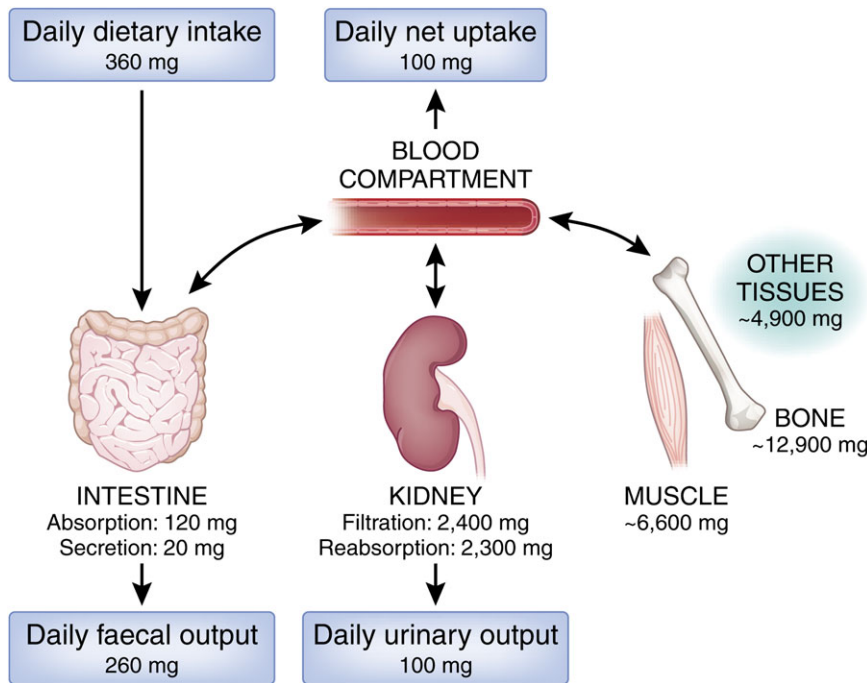
**Absorption pathways.** Two Mg<sup>2+</sup>-absorbing pathways have been identified in the mammalian intestine (Figure 2). Paracellular transport involves the absorption of Mg<sup>2+</sup> through the small spaces between the epithelial cells and is a passive mechanism. Secondly, the transcellular pathway involves the active transport of Mg<sup>2+</sup> to the blood through the interior of the epithelial cell. This second type of Mg<sup>2+</sup> transport is subject to tight regulation since the ions have to pass through two cell membranes.

Paracellular Mg<sup>2+</sup> absorption is responsible for 80–90% of intestinal Mg<sup>2+</sup> uptake. The driving force behind this passive Mg<sup>2+</sup> transport is supplied by the high luminal Mg<sup>2+</sup> concentration, which ranges between 1.0 and 5.0 mmol/L, and the lumen-positive transepithelial voltage of ~+5 mV [2]. Paracellular Mg<sup>2+</sup> absorption relies on tight junction permeability, which is still poorly understood. The ileum and distal parts of the jejunum are known to be the most permeable for ions because of the relatively low expression of 'tightening' claudins 1, 3, 4, 5 and 8 [16]. As such, paracellular Mg<sup>2+</sup> transport seems mainly restricted to these areas that lack the 'tightening' claudins. Claudins 16 and 19, known to be involved in Mg<sup>2+</sup> permeability [17], are not expressed in

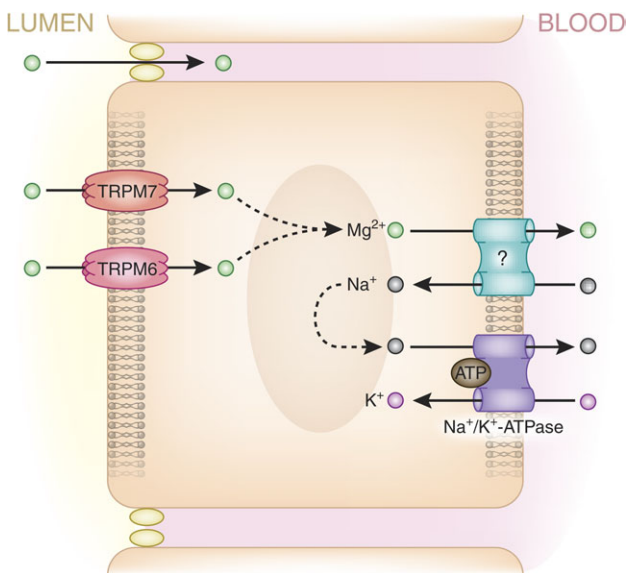
the intestine [16]. The exact mechanism facilitating paracellular Mg<sup>2+</sup> absorption, therefore, remains unknown.

Transient receptor potential channel melastatin member 6 (TRPM6) and TRPM7 Mg<sup>2+</sup> channels mediate transcellular absorption. Whereas TRPM7 is ubiquitously expressed; intestinal TRPM6 expression is mainly detected in the distal small intestine and colon in murine tissue at least, though this result needs to be confirmed in humans [18]. Both TRPM6 and TRPM7 expression is restricted to the luminal membrane of the enterocytes (Figure 2). The basolateral Mg<sup>2+</sup> extrusion mechanism is unknown, but several publications have suggested that basolateral Mg<sup>2+</sup> transport is coupled to the Na<sup>+</sup> gradient, sodium concentrations being lower in the cytoplasm than the blood owing to the action of basolateral Na<sup>+</sup>/K<sup>+</sup>-ATPase [19]. This hypothesis, however, remains to be confirmed by the identification of the basolateral Mg<sup>2+</sup> transporter.

**Regulatory factors.** Intestinal Mg<sup>2+</sup> absorption is regulated by a variety of factors. Mg<sup>2+</sup> absorption is altered by dietary Mg<sup>2+</sup> intake, as demonstrated by Mg<sup>2+</sup> uptake studies [13]. This effect can be attributed, at least partly, to changes in TRPM6 expression in the colon [18]. It also, probably, depends on alterations in paracellular Mg<sup>2+</sup> transport rates owing to changes in the electrochemical gradient.



**Fig. 1.** Magnesium homeostasis. Panels represent the daily amount of  $Mg^{2+}$  intake and excretion. A daily net intake of ~100 mg in the intestine results in a balanced 100 mg excretion in the kidney. In times of  $Mg^{2+}$  shortage, other tissues such as bone and muscle provide  $Mg^{2+}$  to restore blood  $Mg^{2+}$  levels. See also "Magnesium basics" in this supplement [5]. The conversion factor from milligrams to millimole is 0.04113.



**Fig. 2.** A schematic overview of magnesium absorption pathways in the intestine, showing proteins associated with  $Mg^{2+}$  transport in enterocytes. In the intestinal epithelia, paracellular  $Mg^{2+}$  transport occurs via unidentified claudins, occurring concurrently with transcellular  $Mg^{2+}$  transport via transient receptor potential channel melastatin member 6 (TRPM6) and TRPM7 to facilitate  $Mg^{2+}$  absorption.

Furthermore, it has been shown that 1,25-dihydroxyvitamin  $D_3$  [1,25(OH) $_2D_3$ ] can stimulate intestinal  $Mg^{2+}$  absorption [20]. Indeed, patients with chronic renal disease often associated with hypomagnesaemia have low 1,25(OH) $_2D_3$  levels [21, 22]. However, TRPM6 expression in the kidneys is not regulated by 1,25(OH) $_2D_3$  [18]. TRPM6 expression in colon in response to 1,25(OH) $_2D_3$  remains to be determined.

Interestingly, claudins 2 and 12, which are involved in paracellular  $Ca^{2+}$  transport, are regulated by 1,25(OH) $_2D_3$  [23]. As such, one could hypothesize that these claudins are also involved in paracellular  $Mg^{2+}$  absorption. As early as in 1943,  $Mg^{2+}$  absorption was reported to be regulated by protein intake [24]. Fifty years later, this finding was developed when it was demonstrated that it was not  $Mg^{2+}$  absorption but rather intestinal  $Mg^{2+}$  excretion that is altered by high protein intake [25]. Finally, experiments in mice showed that low and high dietary  $Mg^{2+}$  affects  $Ca^{2+}$  balance via the kidney (i.e. increased reabsorption and elimination, respectively) [18]. The mechanisms responsible for these phenomena are unknown, but the authors suggest that a regulatory role for the calcium-sensing receptor (CaSR) could explain the interaction between  $Mg^{2+}$  and  $Ca^{2+}$ .

#### Magnesium storage

While  $Mg^{2+}$  can be stored in muscle fibres, where it plays an important role in the regulation of muscle contraction by antagonizing the action of  $Ca^{2+}$  [26], bone tissue is the largest  $Mg^{2+}$  store in the human body (Figure 1), where it also contributes to the density and strength of the skeleton. Depletion of  $Mg^{2+}$  is, therefore, a risk factor for osteoporosis [27]. A model of  $Mg^{2+}$ -induced bone loss has been proposed in which low blood plasma  $Mg^{2+}$  concentrations lead to activation of bone resorption by osteoclasts and decreased osteoblast bone formation [27]. Moreover, bone surface  $Mg^{2+}$  concentrations (of ~30%) are closely related to serum  $Mg^{2+}$  concentrations, indicating a continuous exchange of  $Mg^{2+}$  between bone and blood [12].

#### Renal magnesium elimination

Approximately 2400 mg of  $Mg^{2+}$  is filtered daily by the glomeruli. Along the nephron, 90–95% of  $Mg^{2+}$  is retrieved;

the remaining 100 mg leaves the body via the urine (Figure 1). The specific roles of the various parts of the nephron are considered in the following sections.

**Proximal tubule.** Surprisingly, little  $Mg^{2+}$  is reabsorbed in the proximal tubule in comparison with other electrolytes such as  $Na^+$ ,  $K^+$  and  $Cl^-$  (Figure 3).  $Mg^{2+}$  concentrations increase as water is reabsorbed, but once a high concentration gradient is obtained then  $Mg^{2+}$  reabsorption occurs via passive paracellular transport, leading to the reabsorption of 10–25% of  $Mg^{2+}$  [28].

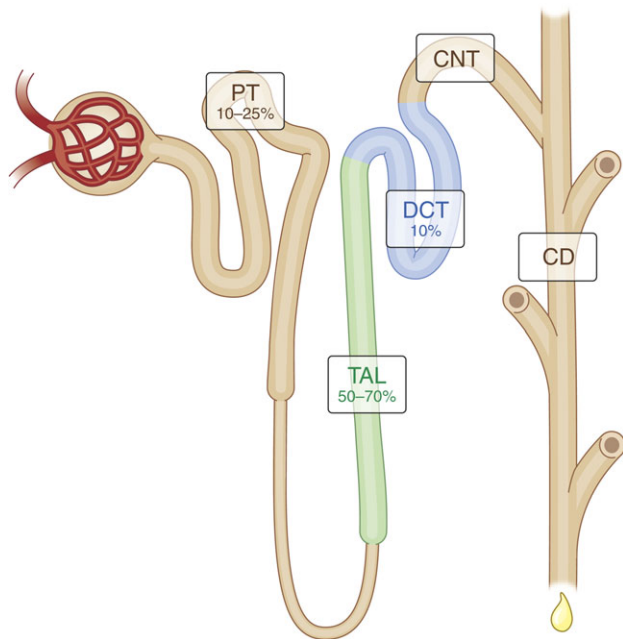
**Thick ascending limb.** The majority of filtered  $Mg^{2+}$  is reabsorbed in the loop of Henle, mostly in the thick ascending limb which accounts for up to 70% of total  $Mg^{2+}$  reabsorption (Figure 3). In fact,  $Mg^{2+}$  is the only bulk-transported ion in the thick ascending limb of the loop of Henle.  $Mg^{2+}$  reabsorption in the proximal tubule and thick ascending limb is, in fact, the opposite to the reabsorption of  $Na^+$  and  $K^+$ , which occurs mainly in the proximal tubule rather than in the thick ascending limb.

The transepithelial voltage gradient is the driving force behind the passive paracellular transport of  $Mg^{2+}$  in the thick ascending limb (voltages in the tubular lumen are positive relative to blood). Furthermore, it has been shown recently that claudins 16 and 19 form a cation-selective tight junction, facilitating the paracellular transport of  $Mg^{2+}$  in the thick ascending limb [17, 29]. The role of these claudins in bulk  $Mg^{2+}$  transport can still be questioned, however, since their  $Mg^{2+}$  transport capacity is fairly low when they are reconstituted in proximal tubule cells [30]. Nevertheless, the current theory proposes that NaCl enters thick ascending loop cells via the apical furosemide-sensitive  $Na^+-K^+-2Cl^-$  cotransporter (NKCC2) (Figure 4).  $K^+$  is recycled into the

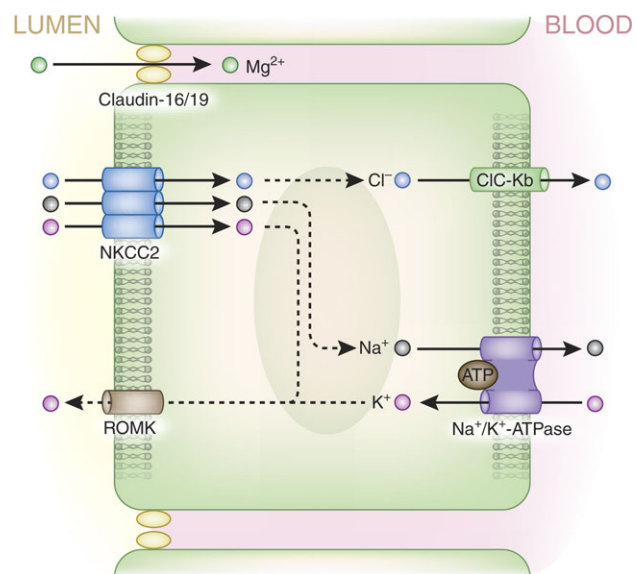
luminal space via the renal outer medullary  $K^+$  (ROMK) channel, whereas  $Na^+$  and  $Cl^-$  are extruded from the cell basolaterally via the  $Na^+/K^+$ -ATPase and the kidney-specific  $Cl^-$  channel, CLC-Kb, respectively. This process establishes the aforementioned lumen-positive potential that drives paracellular  $Mg^{2+}$  transport. It is interesting to note here that inhibition of NKCC2 activity by the use of the diuretic furosemide diminishes this positive charge, can lead to excessive  $Mg^{2+}$  excretion and thus can result in hypomagnesaemia [31, 32].

**Distal convoluted tubule.** The ‘fine-tuning’ of  $Mg^{2+}$  reabsorption takes place along the distal convoluted tubule, where ~10% of filtered  $Mg^{2+}$  is reabsorbed (Figure 3). Here,  $Mg^{2+}$  reabsorption is an active transcellular process that is tightly regulated by several recently discovered factors, each playing an important role in  $Mg^{2+}$  homeostasis [33]. The TRPM6  $Mg^{2+}$  channel allows  $Mg^{2+}$  to enter the cell [34], and while the basolateral  $Mg^{2+}$  extrusion mechanism remains to be identified, it may be dependent on the inward  $Na^+$  gradient that is mediated by the  $Na^+/K^+$ -ATPase (Figure 5). Notably, thiazide diuretics mimic the effects of Gitelman’s syndrome by enhancing  $Na^+$  excretion via inhibition of the  $Na^+-Cl^-$  cotransporter (NCC) (Figure 5). In addition, these drugs are known to affect the  $Mg^{2+}$  balance, inducing hypomagnesaemia, which may be explained by the down-regulation of TRPM6 expression in response to chronic thiazide treatment [35]. Hypomagnesaemia is frequently linked with hypokalaemia owing to disturbances in renal  $K^+$  secretion in the connecting tubule and collecting duct (Figure 3). Low intracellular  $Mg^{2+}$  levels release the  $Mg^{2+}$ -dependent inhibition of ROMK channels, resulting in increased renal  $K^+$  secretion, often leading to hypokalaemia [8].

**Regulatory factors.** Epidermal growth factor (EGF) regulates TRPM6 activity and plasma membrane availability. Interestingly, basolaterally expressed pro-EGF is almost exclusively found in the distal convoluted tubule. Pro-EGF



**Fig. 3.** Magnesium reabsorption along the nephron. The glomerulus filters the blood and facilitates thereby the entrance of  $Mg^{2+}$  into the tubular system that subsequently mediates the reabsorption of 90–95% of  $Mg^{2+}$ . Approximately 10–25% of  $Mg^{2+}$  is reabsorbed in the proximal tubule (PT). Bulk transport (50–70%) of  $Mg^{2+}$  is achieved along the thick ascending limb (TAL) of the loop of Henle. The final  $Mg^{2+}$  concentration in urine is determined in the distal convoluted tubule (DCT) where only 10% of  $Mg^{2+}$  is reabsorbed [28]. CNT, connecting tubule; CD, collecting duct.



**Fig. 4.** Schematic overview of  $Mg^{2+}$  transport pathways in the thick ascending limb of the loop of Henle. The majority of  $Mg^{2+}$  is transported in this part of the nephron.  $Mg^{2+}$  absorption takes place in a paracellular fashion via claudins-16 and -19 of the tight junction complex. The driving force behind  $Mg^{2+}$  transport in the thick ascending limb is the transepithelial voltage gradient.

is cleaved to yield EGF, activating the EGF receptor (EGFR), which in turn triggers an intracellular cascade that regulates TRPM6 activity [36].

Oestrogen is known to stimulate TRPM6 expression [18]. Thus, oestrogen substitution therapy is used to normalize hypermagnesuria, which occurs frequently in post-menopausal women [37]. Interestingly, TRPM6 expression appears to be regulated by plasma  $Mg^{2+}$  levels and oestrogens, but not by  $1,25(OH)_2D_3$  or parathyroid hormone (PTH) action [18].

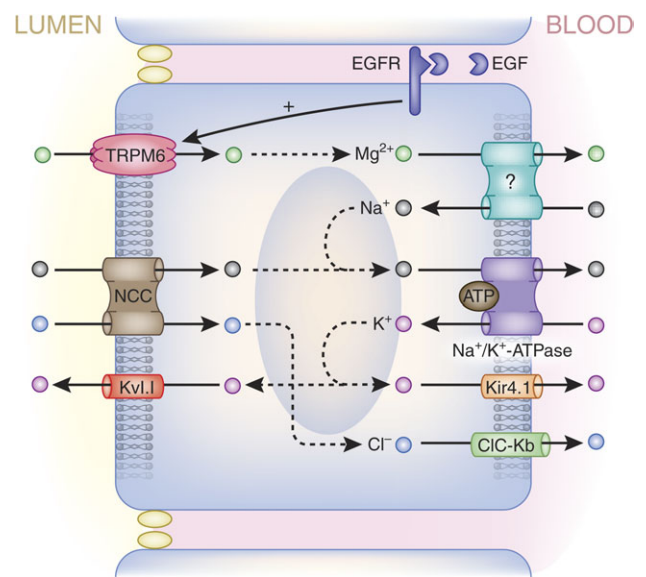
- $Mg^{2+}$  homeostasis depends on three organs: the intestine, facilitating  $Mg^{2+}$  uptake; bone, the main  $Mg^{2+}$  storage system of the body and the kidneys, which are responsible for  $Mg^{2+}$  excretion.
- In the intestine, about 80–90% of  $Mg^{2+}$  is absorbed passively through paracellular transport. The remaining  $Mg^{2+}$  is absorbed via active  $Mg^{2+}$  transporters, which account for the fine-tuning of  $Mg^{2+}$  regulation.
- Bone tissue constitutes the largest  $Mg^{2+}$  store in the human body, though it is also stored in muscle where it acts to antagonize  $Ca^{2+}$  during muscle contraction.
- $Mg^{2+}$  is mainly excreted in the kidney. About 90–95% of daily filtrated  $Mg^{2+}$  is reabsorbed in the kidney. Again, the interplay between passive mechanisms and adjustment via active transporters determine the final  $Mg^{2+}$  concentration.

## Magnesium transporters and genetic disease

In the last decade, gene-linkage studies in families with hereditary forms of hypomagnesaemia have helped identify several proteins involved in renal  $Mg^{2+}$  reabsorption (Table 1). Causative mutations for several genetic disorders have been described and have provided insight in the molecular regulation of  $Mg^{2+}$  transport. In this review, we will present an overview of the implicated diseases and proteins.

### Impaired $Mg^{2+}$ reabsorption in the thick ascending limb

**Claudins 16 and 19.** Claudins are small transmembrane proteins and are regarded as the most important components of the tight junction barrier, which is thought to have a key role in passive paracellular reabsorption (responsible for most  $Mg^{2+}$  reabsorption, which occurs in the thick ascending limb). Mutations in the *claudin-16* gene (formerly known as *paracellin-1* gene) have been shown to be responsible for the rare inherited disorder known as familial hypomagnesaemia with hypercalciuria and nephrocalcinosis (FHHNC) [38]. *Claudin-19* was then identified in Swiss and Spanish families without *claudin-16* mutations, but who suffered from the same symptoms of nephrocalcinosis, progressive renal failure and visual impairment [39]. *Claudin-16* and *claudin-19* colocalize in the thick ascending limb to form a cation-specific complex [29]. This complex is involved in paracellular  $Mg^{2+}$  reabsorption in the thick ascending limb but is also important in voltage-dependent paracellular  $Na^+$  transport (reviewed by Hou and Goode-



**Fig 5.** Ion transport pathways in the distal convoluted tubule. The final possibility of  $Mg^{2+}$  reabsorption is the tightly regulated transcellular transport in the distal convoluted tubule. In this schematic overview, all the proteins whose mutations cause hypomagnesaemia are shown.  $Mg^{2+}$  enters the cell via the TRPM6  $Mg^{2+}$  channel that is regulated by EGF. The Kv1.1  $K^+$  channel maintains transmembrane voltage that is the driving force for  $Mg^{2+}$  transport. The key molecule at the basolateral membrane is the  $Na^+/K^+-ATPase$ , whose expression is regulated by transcription factor HNF1B (not shown). The  $Na^+/K^+-ATPase$  activity is stimulated by its  $\gamma$ -subunit. Kir4.1 is responsible for recycling of  $K^+$  at the basolateral site of the cell. The basolateral  $Mg^{2+}$  transporter remains to be identified. Gitelman's-associated proteins NCC and ClC-Kb are responsible for  $Na^+$  and  $Cl^-$  transport in the distal convoluted tubule.

nough [40]). Knockout models showed that mice deficient in *claudin-16* and *claudin-19* also developed FHHNC [17]. *Claudin-16* and *claudin-19* mutations reduce the cation specificity of the paracellular pathway, diminishing the transepithelial voltage potential. Ultimately, this reduces the driving force for bulk  $Mg^{2+}$  reabsorption in the thick ascending limb and results in renal  $Mg^{2+}$  wasting characteristic of patients with FHHNC [17].

**Barter's syndrome-associated genes.** Barter's syndrome is a group of autosomal-recessive disorders characterized by reduced salt absorption in the thick ascending limb, the target segment of the furosemide diuretics. This salt wasting often coincides with hypokalaemic metabolic alkalosis, elevated renin and aldosterone levels and low blood pressure [7]. Mutations in five different genes have been shown to induce the Barter phenotype [41, 42]. Firstly, loss-of-function mutations in *NKCC2* are responsible for reduced salt reabsorption. The second gene involved encodes the apical  $K^+$  channel ROMK that recycles  $K^+$  into the luminal space. Other gene mutations are those encoding the basolateral  $Cl^-$  channel (CLC-Kb) responsible for basolateral  $Cl^-$  extrusion; Barttin, a protein that regulates CLC-Kb activity and finally, gain-of-function mutations in the *CaSR*, which can cause Barter's syndrome via inhibitory action on *NKCC2* activity. Barter's syndrome is often linked to mild hypomagnesaemia owing to the dissipation of the lumen-positive transepithelial voltage that is the driving force for paracellular  $Mg^{2+}$  transport [43]. Compensatory mechanisms in the distal convoluted tubule may, however, at least partly compensate for the impairment of bulk  $Mg^{2+}$  reabsorption.

### Impaired $Mg^{2+}$ reabsorption in the distal convoluted tubule

*Transient receptor potential channel melastatin member 6 (TRPM6).* Two groups identified *TRPM6*, simultaneously and independently, as the causative gene in the rare genetic disorder of hypomagnesaemia with secondary hypocalcaemia (HSH) [44, 45]. HSH-affected individuals have abnormally low serum  $Mg^{2+}$  levels (<0.40 mmol/L) that indirectly lead to hypocalcaemia, probably because of concomitant parathyroid failure. Patients suffer from neurological symptoms such as seizures and muscle spasms, and eventually, the disorder may be fatal or cause neurological damage if not treated with high-dose  $Mg^{2+}$ .

TRPM6 belongs to the family of transient receptor potential channels that facilitate electrolyte transport. TRPM6 consists of six membrane-spanning domains with a pore-forming region and intracellular C- and N-terminal. In the nephron, TRPM6 expression is limited to the apical membrane of distal convoluted tubule cells, whereas intestinal TRPM6 expression is highest in the colon and caecum [18]. Patch-clamp analysis showed that TRPM6 is a  $Mg^{2+}$ - and  $Ca^{2+}$ -permeable cation channel, which preferentially transports  $Mg^{2+}$  [34]. TRPM6 proteins form homotetrameric functional complexes as well as heterotetrameric complexes with TRPM7, the closest homologue of TRPM6 [46]. Recently, there has been controversy about the necessity of TRPM7 in TRPM6 functioning, as TRPM7-dependent as well as TRPM7-independent TRPM6 activity has been reported [47, 48]. The TRPM6 protein contains a C-terminal  $\alpha$ -kinase domain that seems important in regulatory functions. Interactions between the  $\alpha$ -kinase domain and regulatory factors such as receptor for activated C-kinase 1 (RACK1) and repressor of oestrogen receptor activity (REA) have been shown to modulate TRPM6 activity [49]. TRPM6 knockout mice are lethal, but TRPM6 heterozygous mice survive and have significantly lower plasma  $Mg^{2+}$  levels owing to reduced intestinal and renal expression of TRPM6 [50]. Several TRPM6 loss-of-function mutations have been demonstrated to decrease TRPM6 activity [51]. TRPM6 mutations often affect tetramerization or trafficking to the plasma membrane, but recently, a missense mutation in the pore-forming domain has been reported which leads to a functionally inactive channel [52].

*Epidermal growth factor.* The first reports of the involvement of EGF in the regulation of  $Mg^{2+}$  reabsorption dates from 2005, when symptomatic hypomagnesaemia was observed in a patient with colorectal cancer who was treated with cetuximab, a monoclonal antibody directed against the EGFR [53]. Two years later, EGF was linked to isolated renal hypomagnesaemia (IRH), a rare disorder of low serum  $Mg^{2+}$  concentrations because of renal  $Mg^{2+}$  wasting. Whole-genome linkage analysis and subsequent candidate gene analysis in a family with IRH ( $Mg^{2+}$  serum levels ~0.5 mmol/L) resulted in the identification of mutations in the *EGF* gene [54]. In addition to hypomagnesaemia, the IRH phenotype consists of moderate mental retardation and epileptic seizures. The *EGF* gene encodes a membrane-bound precursor molecule, pro-EGF, which is proteolytically cleaved to release the active form of the EGF hormone. Pro-EGF is expressed both on luminal and basolateral membranes of distal convoluted tubule cells, but EGFR itself is found only on the basolateral membrane.

Hypomagnesaemia was also found as a side effect in a prospective study in colorectal cancer patients given ce-

tuximab therapy, and the authors suggested the involvement of EGF in TRPM6 regulation [55]. Follow-up studies revealed that EGF increases activity and membrane trafficking of TRPM6, and thus, it is the first hormone directly regulating renal  $Mg^{2+}$  reabsorption [56]. The expression of EGF is restricted to the TRPM6-expressing distal convoluted tubule cells. In the kidney, EGF is, therefore, a dedicated hormone regulating the  $Mg^{2+}$  reabsorption in a para- or autocrine fashion. Moreover, Ikari *et al.* [36, 57] reported that EGF regulates TRPM6 transcription via a MEK/ERK/AP-1 pathway, and activation of TRPM6 expression by EGF was confirmed recently *in vivo* in a study with EGFR-inhibitor erlotinib [58]. The mutation that led to the discovery of EGF as magnesiotropic hormone causes impaired basolateral expression of pro-EGF as it disrupts the basolateral-sorting motif. As a consequence, TRPM6 activity and membrane expression are reduced, and thus less  $Mg^{2+}$  is reabsorbed in the distal convoluted tubule.

*Thiazide-sensitive  $Na^+-Cl^-$  cotransporter (NCC).* Gitelman's syndrome is an inherited disorder of impaired salt transport in the distal convoluted tubule and is associated with hypomagnesaemia, hypocalciuria and secondary aldosteronism [7]. Patients generally suffer from tetany, fatigue, chondrocalcinosis and muscle weakness. Gitelman's syndrome is caused by mutations in the thiazide-sensitive  $Na^+-Cl^-$  cotransporter (NCC) gene, resulting in loss of function of the protein. NCC is responsible for apical  $Na^+$  and  $Cl^-$  entry into distal convoluted tubule cells and is sensitive to thiazide blocking [59, 60]. This characteristic of NCC was used in a study investigating the effect of NCC blocking on  $Ca^{2+}$  and  $Mg^{2+}$  reabsorption. The authors showed that thiazide-treated mice have abnormally low expression levels of TRPM6 in the distal convoluted tubule, causing hypomagnesaemia [35]. However, the interesting cross-talk mechanism between NCC activity and TRPM6 expression remains to be resolved.

### Indirect reduction of $Mg^{2+}$ transport in the distal convoluted tubule: transmembrane voltage

*Kv1.1  $K^+$  channel (KCNA1).* Single nucleotide polymorphism-based linkage analysis in a family with autosomal-dominant hypomagnesaemia resulted in the identification of mutations in the *KCNA1* gene, encoding the Kv1.1  $K^+$  channel. Affected individuals have severe hypomagnesaemia (<0.40 mmol/L) and suffer from muscle cramps, tetanic episodes, tremor and muscle weakness. Kv1.1 is a voltage-gated  $K^+$  channel and consists of six transmembrane domains, one of which (S4) acts as the voltage sensor and two (S5/S6) that form the pore region. Immunohistochemistry showed that kidney Kv1.1 localization is restricted to the luminal membrane of distal convoluted tubule cells (Figure 5). In these cells, Kv1.1 is thought to be involved in maintenance of the optimal membrane voltage, which is the driving force behind  $Mg^{2+}$  reabsorption via TRPM6 [61]. Interestingly, the Kv1.1  $K^+$  current is blocked by intracellular  $Mg^{2+}$ , thereby preventing intracellular  $Mg^{2+}$  overload [62]. The N255H mutation, leading to the discovery of the involvement of the *KCNA1* gene in  $Mg^{2+}$  transport, locates in the third membrane-spanning domain and results in a non-functional channel. The asparagine at Position 255 is required for normal channel gating and voltage dependence [63]. It would be interesting to further characterize  $Mg^{2+}$  wasting in Kv1.1 null mice that currently are mainly studied to explain the epileptic seizures they suffer from [64].

**Na<sup>+</sup>/K<sup>+</sup>-ATPase  $\gamma$ -subunit (FXD2).** Patients with dominant renal hypomagnesaemia associated with hypocalciuria suffer from renal Mg<sup>2+</sup> wasting and convulsions. Linkage studies followed by candidate screening led to the discovery of the FXD domain containing the ion transport regulator 2 (FXD2) gene coding for the  $\gamma$ -subunit of the Na<sup>+</sup>/K<sup>+</sup>-ATPase as the causative gene [65]. The basolateral Na<sup>+</sup>/K<sup>+</sup>-ATPase allows the active transport of Na<sup>+</sup> and K<sup>+</sup> transport in the opposite direction (Figure 5). The  $\gamma$ -subunit regulates the kinetics of Na<sup>+</sup>/K<sup>+</sup>-ATPase-mediated exchange of Na<sup>+</sup> and K<sup>+</sup>. Immunohistochemistry confirmed colocalization of the  $\gamma$ - and  $\gamma$ b-subunits at the basolateral membrane of the distal convoluted tubule [66]. Moreover, Na<sup>+</sup>/K<sup>+</sup>-ATPase activity is highest in this part of the nephron [67]. The currently leading hypothesis states that reduced Na<sup>+</sup> transport lowers the membrane potential across the luminal membrane that acts as the inward electrical driving force for Mg<sup>2+</sup>. As a consequence, Mg<sup>2+</sup> reabsorption is reduced. The glycine to arginine mutation at amino acid Position 41 (G41R) found in a Dutch family causes misrouting of the FXD2 complex to the basolateral membrane [65]. G41R-mutated subunits oligomerize with wild-type FXD2, thereby retaining the complex in the cell [68]. A recent study of the G41R mutation proposed a role for FXD2 as an inward rectifying channel. These findings resulted in the suggestion that FXD2 mediates basolateral Mg<sup>2+</sup> extrusion [69]. Future studies, however, need to address the exact role of the  $\gamma$ -subunit of the Na<sup>+</sup>/K<sup>+</sup>-ATPase in the distal convoluted tubule.

**Hepatocyte nuclear factor 1 homeobox B (HNF1B).** Mutations in the HNF1B transcription factor are associated with many human disorders such as neonatal diabetes and renal malformation. Recently, HNF1B mutations were linked to hypomagnesaemia in a 13-year-old Pakistani boy and a cohort of patients with HNF1B mutations and renal malformations. Forty-four per cent of the HNF1B mutation carriers have hypomagnesaemia, hypermagnesuria and hypocalciuria [70]. Luciferase reporter assays demonstrated that HNF1B stimulates transcriptional expression of the FXD2a gene [66]. Mutations in HNF1B prevented transcriptional activation of the  $\gamma$ -subunit of the Na<sup>+</sup>/K<sup>+</sup>-ATPase, underlining the importance of the Na<sup>+</sup>/K<sup>+</sup>-ATPase in renal Mg<sup>2+</sup> reabsorption.

**Kir4.1 K<sup>+</sup> channel (KCNJ10).** Quantitative trait loci mapping studies of seizure-sensitive mice led to the nomination of KCNJ10 as the responsible gene [71]. Recently, two groups confirmed that hypomagnesaemia (~0.6 mmol/L) associated with seizures, sensorineural deafness, ataxia, mental retardation and electrolyte imbalance (SeSAME syndrome, also referred to as EAST) is provoked by mutations in the KCNJ10 gene, which encodes the Kir4.1 K<sup>+</sup> channel [72, 73]. Kir4.1 is expressed in glial cells, epithelium of the inner ear and the basolateral side of kidney distal convoluted tubule cells, where it is involved in K<sup>+</sup> recycling necessary for optimal Na<sup>+</sup>/K<sup>+</sup>-ATPase activity [74] (Figure 5). By this mechanism, it is indirectly involved in the regulation of the intracellular voltage that is required for Mg<sup>2+</sup> transport, explaining the hypomagnesaemia observed in patients with Kir4.1 mutations.

Kir4.1 and the CaSR have recently been shown to physically interact in human embryonic kidney cells and in kidney homogenates, and the CaSR appears to regulate Kir4.1 activity by decreasing Kir4.1 membrane availability via a G $\alpha$ - and caveolin-dependent pathway [75]. A Kir4.1 knockout

mouse model is available, but no data on kidney Mg<sup>2+</sup> transport from this model has been published. The Kir4.1<sup>-/-</sup> mice have severe neurological problems and die prematurely (in the first few weeks after birth) [76, 77]. Several Kir4.1 mutations identified in SeSAME syndrome patients have been studied, and although all mutations modified the channel function, the mechanisms underlying these disturbances are different. Some mutations resulted in a shift in pH sensitivity causing changes in pore gating, while others impaired correct protein folding and decreased surface expression [78].

- Studies of genetic human diseases associated with hypomagnesaemia have extended our understanding of Mg<sup>2+</sup> reabsorption in the nephron.
- Claudins are thought to have a key role in passive paracellular reabsorption in the thick ascending limb.
- Active transcellular Mg<sup>2+</sup> reabsorption occurs in the distal convoluted tubule, where TRPM6 has been identified as the luminal Mg<sup>2+</sup> channel. The process is regulated by a variety of factors, including EGF.
- Gitelman's-associated proteins NCC and ClC-Kb are responsible for Na<sup>+</sup> and Cl<sup>-</sup> transport in the distal convoluted tubule. The disease is associated with hypomagnesaemia caused by a low TRPM6 expression.
- Kv1.1 K<sup>+</sup> channel maintains the apical transmembrane voltage, thought to be the driving force behind Mg<sup>2+</sup> reabsorption via TRPM6 in the distal convoluted tubule.
- The key molecule at the distal convoluted tubule basolateral membrane is the Na<sup>+</sup>/K<sup>+</sup>-ATPase, whose expression is regulated by transcription factor HNF1B.
- Kir4.1 is responsible for recycling of K<sup>+</sup> at the basolateral site of the cell and so is indirectly involved in intracellular voltage regulation needed for Mg<sup>2+</sup> transport.

## Conclusions and future perspectives

Mg<sup>2+</sup> plays a vital physiological role in the body, and therefore control of plasma Mg<sup>2+</sup> level is of major importance. Mg<sup>2+</sup> homeostasis depends on its uptake in the intestine, storage in bone tissue and its excretion by the kidneys. When Mg<sup>2+</sup> intake is low, its absorption can rise from 30 to 50% of dietary Mg<sup>2+</sup> to ~80%. Within the nephron, filtered Mg<sup>2+</sup> is mainly reabsorbed in the loop of Henle, particularly in the thick ascending limb. However, the 'fine-tuning' of Mg<sup>2+</sup> reabsorption occurs along the distal convoluted tubule, where ~10% of filtered Mg<sup>2+</sup> is reabsorbed in a tightly regulated and active transcellular process.

During the last decade, studies of human diseases have helped to extend the understanding of Mg<sup>2+</sup> reabsorption in the nephron. In the thick ascending limb, claudins 16

and 19 form the pore permitting paracellular  $Mg^{2+}$  reabsorption, a process which depends on the transepithelial voltage which is maintained by transcellular salt transport. Active transcellular  $Mg^{2+}$  reabsorption takes place in the distal convoluted tubule, where TRPM6 has been identified as the luminal  $Mg^{2+}$  channel, and it is regulated by a variety of factors (of which EGF is the best studied). Pro-EGF is cleaved from the distal convoluted tubule cell, releasing EGF, where it can activate the EGFR at the basolateral membrane. TRPM6 can form homotetrameric complexes and heterotetrameric complexes with TRPM7; however, the necessity of TRPM7 for TRPM6 functioning is controversial. TRPM6 activity is dependent on the transmembrane voltage that is kept intact by the luminal  $K^+$  channel Kv1.1, but the exact role of Kv1.1 has to be further examined.

The basolateral  $Mg^{2+}$  extrusion mechanism is less well understood but is known to be linked to the basolateral  $Na^+/K^+$ -ATPase.  $Na^+/K^+$ -ATPase activity is regulated by the HNF1B transcriptional factor, which stimulates the expression of FXD2. Also, the Kir4.1  $K^+$  channel is involved in the  $Na^+/K^+$ -ATPase activity since it facilitates the availability of  $K^+$  ions. The involvement of the  $Na^+/K^+$ -ATPase in transcellular  $Mg^{2+}$  transport is poorly understood. Many genes of transporters and regulatory proteins have been identified, but several questions remain unanswered. A review summarizing these newly identified magnesiumotropic proteins has been recently published [79]. Most importantly, the basolateral  $Mg^{2+}$  extrusion mechanism still has to be identified. Recently, patients with hypomagnesaemia have been screened for several candidate genes, but without result. New generation DNA sequencing techniques may contribute to the final identification of the missing transporter.

$Mg^{2+}$  reabsorption in the distal convoluted tubule is tightly regulated by plasma  $Mg^{2+}$  levels [18], suggesting the existence of a  $Mg^{2+}$ -sensing mechanism. The pathway that is involved in this mechanism, regulating for instance TRPM6 expression, may be discovered in the near future. The unidentified protein involved in  $Mg^{2+}$ -sensing might be regulated by hormones since it has been shown that  $1,25(OH)_2D_3$  regulates intestinal  $Mg^{2+}$  absorption [20]. Next to  $1,25(OH)_2D_3$ , several other factors, such as PTH or oestrogen, might be involved.

Genetic research in families with hypomagnesaemia has expanded our understanding of renal  $Mg^{2+}$  reabsorption. Understanding these processes can lead to new therapies for chronic hypomagnesaemia, and new state-of-the-art DNA screening techniques can help identify the missing magnesiumotropic genes. Recent developments in renal  $Mg^{2+}$  research are excellent examples of bedside-to-bench research cooperation between clinicians and fundamental researchers and continuation of these collaborations may also allow new insights into the sequence of events that occurs when chronic renal failure develops.

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