The Nutrition Society Winter Conference Live 2020 was held virtually on 8–9 December 2020

Conference on 'Micronutrient malnutrition across the life course, sarcopenia and frailty'
Symposium three: Ageing, frailty, sarcopenia, osteoporosis and micronutrients

Genetic and drug-induced hypomagnesemia: different cause, same mechanism

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Magnesium (Mg^{2+}) plays an essential role in many biological processes. Mg^{2+} deficiency is therefore associated with a wide range of clinical effects including muscle cramps, fatigue, seizures and arrhythmias. To maintain sufficient Mg^{2+} levels, (re)absorption of Mg^{2+} in the intestine and kidney is tightly regulated. Genetic defects that disturb Mg^{2+} uptake pathways, as well as drugs interfering with Mg^{2+} (re)absorption cause hypomagnesemia. The aim of this review is to provide an overview of the molecular mechanisms underlying genetic and drug-induced Mg^{2+} deficiencies. This leads to the identification of four main mechanisms that are affected by hypomagnesemia-causing mutations or drugs: luminal transient receptor potential melastatin type 6/7-mediated Mg^{2+} uptake, paracellular Mg^{2+} reabsorption in the thick ascending limb of Henle's loop, structural integrity of the distal convoluted tubule and Na^+-dependent Mg^{2+} extrusion driven by the Na^+/K^+-ATPase. Our analysis demonstrates that genetic and drug-induced causes of hypomagnesemia share common molecular mechanisms. Targeting these shared pathways can lead to novel treatment options for patients with hypomagnesemia.

Magnesium: Ion transport: Genetic diseases: Drug side effects

Magnesium (Mg^{2+}) is a crucial micronutrient present in foods such as nuts, grains, seeds and vegetables. The recommended daily intake of Mg^{2+} is 420 mg for men and 320 mg for women[1]. Mg^{2+} is the second most abundant cation intracellularly where it is involved in many biological processes, including hundreds of enzymatic reactions, cell signalling and DNA/RNA synthesis[2]. Mg^{2+} homeostasis therefore has to be tightly maintained. In the gastrointestinal tract, the majority of Mg^{2+} absorption occurs paracellularly in the small intestine, while in the colon and cecum, Mg^{2+} is absorbed via a transcellular pathway[3]. Normally, 30–50 % of dietary Mg^{2+} is absorbed, but this can be increased to 80 % when intake is low[4]. In the kidney, Mg^{2+} is filtered into the pro-urine, from which 95–99 % is reabsorbed in different segments of the nephron[5]. In total, 10–25 % of Mg^{2+} reabsorption occurs in the proximal tubule and 50–70 % in the thick ascending limb of Henle's loop (TAL), both via a paracellular pathway. The remaining 5–10 % is reabsorbed transcellularly in the

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distal convoluted tubule (DCT), where the final urinary Mg^{2+} concentration is determined as this is the last segment where Mg^{2+} can be reabsorbed.

When the intestinal and renal regulatory mechanisms aimed at maintaining sufficient Mg^{2+} levels are disturbed, hypomagnesemia (serum Mg^{2+} <0·7 mM) can develop. Consistent with the widespread functions of Mg^{2+}, severe symptoms such as epilepsy, fatigue, muscle cramps and cardiac arrhythmias have been observed as a consequence of hypomagnesemia[2]. Various factors can cause hypomagnesemia. Low dietary Mg^{2+} intake is a growing problem, caused by unhealthy diets and a decreased Mg^{2+} content in soil and foods[6]. Other causes of hypomagnesemia are alcoholism, mutations in genes involved in Mg^{2+} regulation or certain drug treatments[2]. Genetic and drug-induced Mg^{2+} deficiencies in particular have contributed greatly to our understanding of Mg^{2+} homeostasis. Since the number of hypomagnesemia-causing mutations and drugs is steadily growing, the underlying mechanisms are becoming more elaborate and complex. Studying how these mechanisms are connected can help with finding the most important contributors to Mg^{2+} maintenance. Therefore, the aim of this review is to provide an up-to-date overview of the molecular causes of genetic and drug-induced Mg^{2+} deficiencies and identify the main pathways of Mg^{2+} regulation shared between them.

Genetic causes of hypomagnesemia

The development of novel sequencing techniques has resulted in the identification of various genes that play a role in Mg^{2+} homeostasis (Table 1). In the following segment, the genetic Mg^{2+} deficiencies are subdivided into groups that share a common mechanism.

Luminal Mg^{2+} uptake in the intestine and distal convoluted tubule

Transient receptor potential melastatin type 6 (TRPM6) is a divalent cation transporter with a high affinity for Mg^{2+} that is specifically expressed in the luminal/apical membranes of the colon and DCT[7]. Mutations in the *TRPM6* gene cause severe hypomagnesemia with secondary hypocalcemia, which occurs because of hypomagnesemia-induced parathyroid failure[8,9]. There is some debate on whether hypomagnesemia is caused by intestinal Mg^{2+} malabsorption or renal Mg^{2+} wasting. Kidney-specific TRPM6 knockout mice display normal Mg^{2+} levels[10]. However, in patients with *TRPM6* mutations, intestinal malabsorption, as well as increased urinary Mg^{2+} excretion, is observed, suggesting both intestinal and renal mechanisms are involved[11]. TRPM6 is able to form a heteromeric complex with its family member TRPM7, which is essential for TRPM6 to facilitate Mg^{2+} transport[10,12]. In contrast to TRPM6, TRPM7 is ubiquitously expressed and is able to transport Mg^{2+} and other divalent cations by itself[13]. Consequently, TRPM7 deficiency usually decreases intracellular Mg^{2+} content[10,14]. In TRPM6-expressing

colon cells, however, TRPM7 down-regulation actually increased Mg^{2+} influx[15]. This may be explained by the fact that in these cells, down-regulation of TRPM7 increases the relative abundance of TRPM6/7 heteromers compared to TRPM7 monomers. TRPM6/7 heteromers show higher Mg^{2+} currents than TRPM7 monomers and are less sensitive to inhibition by the intracellular Mg^{2+} concentration[16]. Thus, TRPM7 by itself provides an adaptable Mg^{2+} transport throughout the body, while TRPM6/7 complexes facilitate a high and constitutive Mg^{2+} uptake in the intestine and DCT that is needed to maintain sufficient Mg^{2+} levels. Because of the ubiquitous, TRPM6-independent role of TRPM7 in the maintenance of the cellular cation balance, it remains to be seen whether *TRPM7* mutations can cause a phenotype similar to *TRPM6* mutations.

Epidermal growth factor (EGF) is an essential regulator of TRPM6 activity[17]. *EGF* and EGF receptor *(EGFR)* mutations cause hypomagnesemia along with either mental retardation *(EGF)* or severe epithelial inflammation *(EGFR)*[18,19]. Within the kidney, EGF is predominantly expressed in the DCT[18]. In human embryonic kidney 293 cells transiently transfected with TRPM6, EGF dose-dependently increased TRPM6 activity[18]. This effect is mediated by EGFR-activated Src family kinases, which in turn activate the PI3K/Akt pathway[17]. Upon activation of this pathway, the downstream GTPase Rac1 increases the surface expression of TRPM6 (Fig. 1a). Recent studies have demonstrated that the EGFR directly binds to TRPM7 in the vasculature to regulate Mg^{2+} uptake[20]. Whether this mechanism also contributes to Mg^{2+} uptake in the DCT and colon is unknown, though this is unlikely considering the localisations of TRPM6/7 and EGFR in these tissues are apical and basolateral, respectively. As EGF signalling is classically studied in cell differentiation and organ development[21], the biological function of this regulatory pathway is unclear. If EGF would act as an Mg^{2+}-regulating hormone, its release should depend on Mg^{2+} availability similar to parathyroid hormone for Ca^{2+} homeostasis[22]. In mice fed an Mg^{2+}-deficient diet, EGF up-regulation has been reported specifically in DCT cells[23], indicating Mg^{2+}-dependent EGF transcription may occur locally in the DCT. Alternatively, EGF-induced Mg^{2+} uptake may contribute to the growth factor function of EGF, as cell growth requires high intracellular Mg^{2+} concentrations for transcription and translation. Several studies indeed report up-regulation of EGF and TRPM7 in cancer to facilitate rapid Mg^{2+} uptake and cell growth[24–27].

Paracellular Mg^{2+} reabsorption in the thick ascending limb of Henle's loop

The TAL is responsible for the majority of Mg^{2+} reabsorption via a passive paracellular pathway[28]. This paracellular transport is enabled by cation-selective pores in tight junction complexes formed by claudin-16 and claudin-19, encoded by *CLDN16* and *CLDN19*[29]. By disrupting this complex, mutations in *CLDN16* and *CLDN19* cause familial hypomagnesemia, hypercalciuria and nephrocalcinosis with ocular abnormalities in the

Table 1. Mechanisms of genetic Mg^{2+} deficiencies

Mechanism	Gene	Protein	Refs
TRPM6/7-mediated Mg^{2+} uptake	TRPM6	TRPM6	[8,9]
	EGF	EGF	[18]
	EGFR	EGFR	[19]
Paracellular Mg^{2+} reabsorption in the TAL	CLDN16	Claudin-16	[30]
	CLDN19	Claudin-19	[31]
	CASR	CaSR	[39,40]
Structural integrity of the DCT	SLC12A3	NCC	[44]
Mg^{2+} extrusion driven by the Na^+/K^+-ATPase	ATP1A1	α1 subunit of the Na^+/K^+-ATPase	[54]
	FXYD2	γ subunit of the Na^+/K^+-ATPase	[55]
	KCNJ10	Kir5·1	[59,60]
	CLCNKB	ClC-Kb	[67]
	HNF1B	HNF1β	[68,70]
	PCBD1	PCBD1	[69]
	Mitochondrial genes	Seryl tRNA synthetase, POLG1	[73–76]
Unclear function	CNNM2	Cyclin M2	[77–81]
	FAM111A	FAM111A	[85,86]
	GATA3	GATA3	[87]
	PLVAP	PLVAP	[88]

TRPM6/7, transient receptor potential melastatin type 6/7; EGF(R), epidermal growth factor (receptor); TAL, thick ascending limb of Henle's loop; CaSR, calcium-sensing receptor; DCT, distal convoluted tubule; NCC, Na^+, Cl^- co-transporter; Kir5·1, K^+ inwardly rectifying channel 5·1; ClC-Kb, Cl^- channel Kb; HNF1β, hepatocyte nuclear factor 1β; PCBD1, pterin-4α-carbinolamine dehydratase; POLG1, DNA polymerase subunit gamma; FAM111A, family with sequence similarity 111 member A; PLVAP, plasmalemma vesicle-associated protein.

case of *CLDN19*[30,31]. Claudin-16 also interacts with claudin-14, which impairs the cation permeability of the tight junction complex and thus serves as a negative regulator of the claudin-16/claudin-19 channel (Fig. 1b)[32].

The calcium-sensing receptor (CaSR) is an important regulator of claudin-mediated paracellular reabsorption. In the kidney, the CaSR is highly abundant on the basolateral membrane of the TAL[33,34]. The protein promotes Ca^{2+} excretion when Ca^{2+} concentrations in the blood are high[35], by inhibiting claudin-16 and promoting claudin-14 expression, thereby limiting paracellular transport in the TAL[32,36]. Moreover, CaSR activation inhibits the renal outer medullary potassium channel and the Na^+, K^+ and $2Cl^-$ co-transporter (NKCC2) on the apical membrane of the TAL[37]. Renal outer medullary potassium channel and NKCC2 play an important role in generating a lumen-positive voltage which allows the paracellular transport of cations[38]. By inhibiting renal outer medullary potassium channel and NKCC2 and activating claudin-14, gain-of-function mutations in *CASR* lead to autosomal-dominant hypocalcaemia and hypomagnesemia[39,40]. Not all autosomal-dominant hypocalcaemia patients develop hypomagnesemia, however, as this is dependent on the level of activity of the mutant CaSR[41]. This indicates that relatively mild wasting of Mg^{2+} in the TAL will not cause Mg^{2+} deficiency, probably because this can be compensated by an increased Mg^{2+} reabsorption in the DCT. Indeed, inhibition of ion transport in the TAL leads to the proliferation of the DCT and an increased expression of TRPM6[42,43].

Structural integrity of the distal convoluted tubule

Mutations in *SLC12A3*, encoding the thiazide-sensitive Na^+ and Cl^- co-transporter (NCC), cause Gitelman syndrome, one of the most common inherited renal disorders[44]. Although NCC transports Na^+ and Cl^-, hypomagnesemia and hypokalaemia are the main symptoms of Gitelman syndrome. In a recent review, the link between NCC activity and Mg^{2+} transport was extensively discussed[45]. The most commonly accepted hypothesis is that NCC deficiency causes atrophy of the DCT segment (Fig. 1c)[46]. This is in line with a reduction in the expression of the DCT marker parvalbumin and TRPM6 upon inactivation of NCC in mice[46,47]. The hypothesis of DCT atrophy is supported by other studies showing that the DCT has a high degree of plasticity and can remodel depending on the situation[42,48–50]. Recently, the transcription factor AP-2β and its downstream target potassium channel tetramerisation domain containing 1 (KCTD1) were identified as regulators of DCT development[51]. Similar to the DCT atrophy observed in NCC knockout mice, impaired development of the DCT as a consequence of KCTD1 deficiency also leads to hypomagnesemia[52]. Clearly, proper development of DCT structure and function under the influence of genes such as *SLC12A3* and *KCTD1* is crucial for Mg^{2+} homeostasis. Although no hypomagnesemia-causing variants are known in the *KCTD1* gene, it could be an interesting candidate to consider in the analysis of unsolved cases.

Na^+-dependent extrusion of Mg^{2+} driven by the Na^+/K^+-ATPase

Although the exact molecular identity of the basolateral Mg^{2+} extrusion protein in the DCT is under debate, it is generally accepted that Mg^{2+} extrusion is Na^+-dependent[53]. This notion is further supported by the identification of hypomagnesemia-causing mutations in two genes encoding subunits of the Na^+/K^+-ATPase, *ATP1A1* and *FXYD2*[54,55]. The Na^+/K^+-ATPase
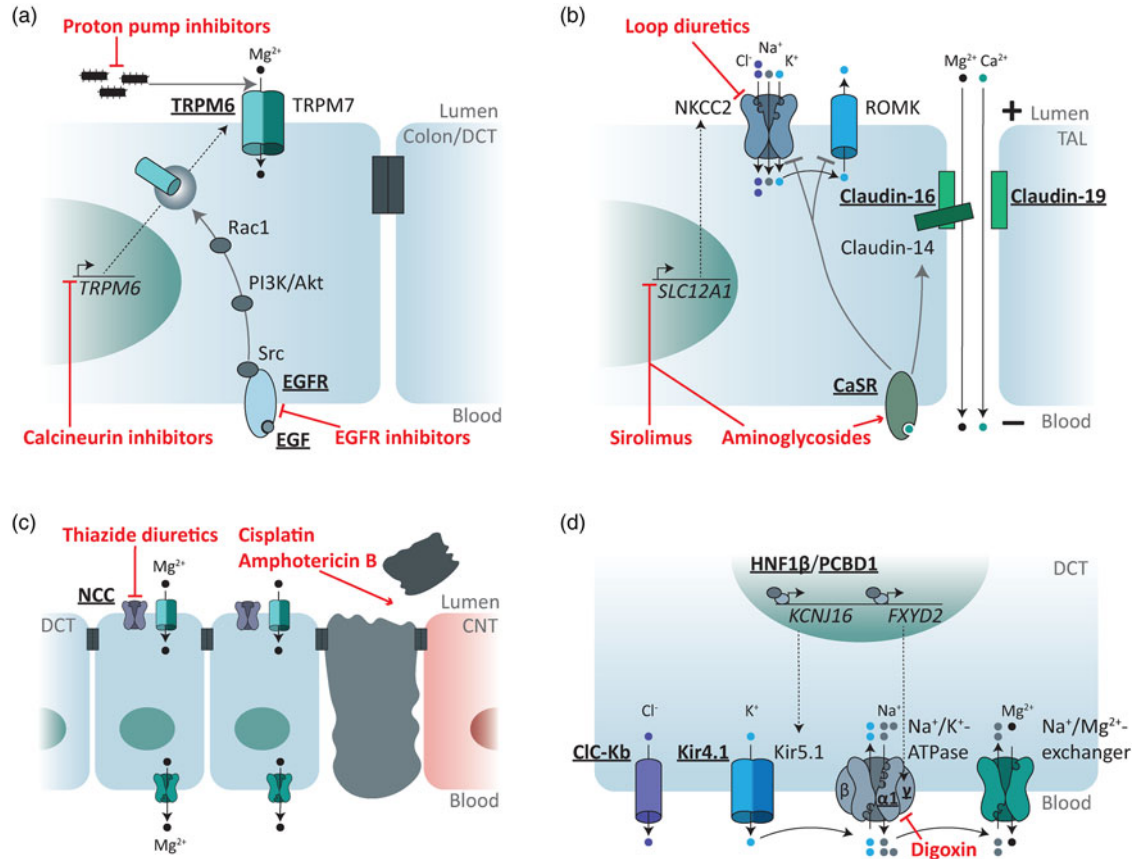
Fig. 1. Main molecular mechanisms affected in genetic and drug-induced hypomagnesemia. Proteins in which mutations are associated with hypomagnesemia are underlined and highlighted in bold, hypomagnesemia-causing drugs are highlighted in red. (a) In the colon and DCT, TRPM6/7 heteromers facilitate efficient (re)absorption of Mg^{2+} from the lumen. EGF signalling increases TRPM6 trafficking to the membrane. EGFR and calcineurin inhibitors decrease the (membrane) expression of TRPM6. The effects of the microbiota and PPI are specific to the colon. (b) In the TAL, Mg^{2+} is transported paracellularly through pores formed by claudin-16 and -19 and blocked by CaSR-activated claudin-14. The required lumen-positive voltage is generated by NKCC2 and ROMK. Drugs that inhibit NKCC2 or activate CaSR decrease Mg^{2+} reabsorption. (c) DCT length is crucial for sufficient Mg^{2+} reabsorption. NCC deficiency or nephrotoxic drugs can cause DCT atrophy. (d) Mg^{2+} is extruded through a putative Na^+/Mg^{2+}-exchanger driven by the Na^+/K^+-ATPase. Extrusion of K^+ through Kir4·1/Kir5·1 channels is required for Na^+/K^+-ATPase function and Cl^- transport through ClC-Kb. Expression of Kir 5·1 and the γ-subunit of the Na^+/K^+-ATPase is activated by HNF1β and PCBD1. CaSR, calcium-sensing receptor; ClC-Kb, Cl^- channel Kb; CNT, connecting tubule; DCT, distal convoluted tubule; EGF(R), epidermal growth factor (receptor); HNF1β, hepatocyte nuclear factor 1β; Kir4·1/5·1, K^+ inwardly rectifying channel 4·1/5·1; NCC, Na^+, Cl^- co-transporter; NKCC2, Na^+, K^+, $2Cl^-$ co-transporter; PCBD1, pterin-4α-carbinolamine dehydratase; PPI, proton pump inhibitors; ROMK, renal outer medullary potassium channel; TAL, thick ascending limb of Henle's loop; TRPM6/7, Transient receptor potential melastatin type 6/7.

consists of an α-, a β- and a regulatory FXYD/γ-subunit and crucially maintains favourable electrochemical gradients in all cells of the body by exchanging three Na^+ ions for two K^+ ions using ATP[56]. Interestingly, hypomagnesemia is associated with seizures and intellectual disability in the case of *ATP1A1*[54]. However, it is unclear whether this is a secondary effect of hypomagnesemia, or indicates an intrinsic function of ATP1A1 in the brain. Based on animal models, the most abundant expression of *ATP1A1* and *FXYD2* proteins within the kidney is in the DCT[57,58]. Consequently, two mechanisms can be proposed for the hypomagnesemia in patients with *ATP1A1* and *FXYD2* mutations. First,

the Na^+/K^+-ATPase may generate a favourable electrochemical gradient for transcellular Mg^{2+} transport in the DCT. As such, its function is essential for TRPM6-mediated Mg^{2+} entry into the cell[54]. Secondly, the basolateral Na^+ gradients may be essential for the function of the Na^+/Mg^{2+}-exchanger, which is driven by extracellular Na^+ (Fig. 1d)[53]. The relative contribution of both pathways to the development of hypomagnesemia is unclear. Interestingly, *ATP1A1* and *FXYD2* patients have a relatively mild Na^+-wasting phenotype and often do not display hypokalaemia or metabolic alkalosis[54,55]. If Na^+ reabsorption in the DCT is decreased, downstream segments will compensate for this by

reabsorbing Na$^+$ at the expense of K$^+$ and H$^+$ excretion, resulting in hypokalaemia and metabolic alkalosis. Since these phenotypes are not present, it seems that sufficient Na$^+$/K$^+$-ATPase function remains to maintain NCC-mediated Na$^+$ reabsorption in the DCT. This suggests that Mg^{2+} reabsorption is more sensitive to disturbed Na$^+$/K$^+$-ATPase function than Na$^+$.

In line with reduced Na$^+$/K$^+$-ATPase function, mutations in *KCNJ10* cause EAST/SeSAME syndrome, characterised by epilepsy, ataxia, sensorineural deafness and a tubulopathy[59,60]. Patients suffer from a Gitelman syndrome-like electrolyte phenotype including hypomagnesemia, hypokalaemia and metabolic alkalosis. *KCNJ10* encodes the K$^+$ inwardly rectifying channel 4·1 (Kir4·1), which together with its binding partner Kir5·1 (encoded by *KCNJ16*) forms the major K$^+$ channel in the basolateral membrane of the TAL and DCT[61,62]. These Kir4·1/Kir5·1 channels provide the driving force for Na$^+$/K$^+$-ATPase by recycling K$^+$ at the basolateral membrane (Fig. 1d). Mutations in Kir4·1 will therefore limit the functionality of Na$^+$/K$^+$-ATPase, explaining the hypomagnesemia[63]. Moreover, uncoupling of this 'pump-leak mechanism' at the basolateral membrane will result in plasma membrane depolarisation. As a result, the Cl$^-$ extrusion via the kidney-specific basolateral Cl$^-$ channel Kb will be decreased, leading to an increased intracellular Cl$^-$ concentration[64]. As Cl$^-$ inhibits the NCC-activating with-no-lysine kinases[65,66], NCC-mediated Na$^+$ reabsorption will be decreased. The downstream compensatory mechanism of Na$^+$ reabsorption at the expense of K$^+$ and H$^+$ thus explains the hypokalaemia and metabolic alkalosis. In accordance with this mechanism, mutations in *CLCNKB*, which encodes for Cl$^-$ channel Kb, are associated with a similar phenotype of Na$^+$ and K$^+$ wasting and metabolic alkalosis[67]. Hypomagnesemia can also be observed in these patients, which is most likely due to the link between impaired NCC functioning and Mg^{2+} reabsorption.

Expression of *FXYD2* is activated by the transcription factor hepatocyte nuclear factor 1β (HNF1β) and its coactivator pterin-4α-carbinolamine dehydratase (PCBD1)[68,69]. Mutations in both *HNF1B* and *PCBD1* are associated with hypomagnesemia[68–70]. These mutations abolish the HNF1β/PCBD1-induced transcription of *FXYD2*, confirming that *FXYD2* plays an important role in Mg^{2+} homeostasis[69,71]. Importantly, HNF1β also regulates *KCNJ16* transcription[72]. Down-regulation of HNF1β indeed reduces the expression of Kir5·1 as well as Kir4·1 and NCC[72]. This indicates that *HNF1B* mutations affect the Na$^+$/K$^+$-ATPase directly via *FXYD2*, but also less directly via Kir4·1/Kir5·1. Furthermore, it should be noted that HNF1β has many other target genes. Therefore, it cannot be excluded that additional targets play a role in disturbed Mg^{2+} homeostasis associated with *HNF1B* mutations. In line with this broader function, *HNF1B* mutations typically lead to widespread abnormal renal development of which hypomagnesemia is only one of the manifestations.

Several mutations in mitochondrial DNA or in genes encoding mitochondrial proteins have been associated with hypomagnesemia[73–76]. Although the mechanisms of these disorders have never been examined, the essential role of the Na$^+$/K$^+$-ATPase in Mg^{2+} transport may partially explain the phenotype. Since the energy demand of Na$^+$/K$^+$-ATPase is particularly high in the DCT, optimal ATP production by the mitochondria is essential to fuel its activity. Indeed, mitochondrial density is very high in the DCT[77]. However, as mitochondrial mutations often lead to variable phenotypes due to differences in affected tissues and heteroplasmy levels, it is particularly challenging to unravel how Mg^{2+} homeostasis is affected in these patients. Therefore, it should be noted that additional pathways may be involved.

Others

Not all genes in which hypomagnesemia-causing mutations have been found can already be placed in major Mg^{2+} regulatory pathways as described earlier. Mutations in the cyclin M2 gene *CNNM2* cause hypomagnesemia, seizures, intellectual disability and obesity[78–81]. Therefore, CNNM2 likely plays a role in brain development and metabolism as well as Mg^{2+} balance[79,81]. There is a debate on whether CNNM2 and its family members CNNM1, CNNM3 and CNNM4 are the putative Na$^+$/Mg^{2+}-exchangers responsible for Mg^{2+} extrusion[82,83]. The idea is supported by the fact that CNNM2 is highly expressed in the DCT, localises to the basolateral membrane, and stimulates Mg^{2+} efflux and Na$^+$ influx when overexpressed[78,82,84]. Conversely, CNNM2-induced Mg^{2+} transport may be bi-directional, is not always shown to be Na$^+$ dependent and is abolished by TRPM7 inhibition, indicating CNNM2 itself is not a transporter[78,79]. An explanation that seems to fit all observations is that CNNM2 has a regulatory rather than a transporting function, though its exact function and the molecular identity of the Na$^+$/Mg^{2+}-exchanger remains to be determined.

Other genes with an unsolved function in Mg^{2+} homeostasis include *FAM111A*, in which specific mutations cause hypomagnesemia along with bone and eye abnormalities and hypoparathyroidism[85,86]. Its known functions in antiviral restriction and DNA replication cannot be clearly linked to Mg^{2+}, so efforts to unravel this are ongoing. Mutations in *GATA3*, encoding a transcription factor, typically lead to hypoparathyroidism, deafness and renal anomalies, but hypomagnesemia has also been reported[87]. It is unknown whether this Mg^{2+} deficiency is a sporadically occurring secondary effect of the syndrome or if GATA3 regulates the expression of genes that are of interest for Mg^{2+} transport. Lastly, Mg^{2+} deficiency has also been observed in a patient with protein-losing enteropathy as a consequence of mutations in the plasmalemma vesicle-associated protein gene, which is expressed in the endothelium[88]. The hypomagnesemia may occur as a consequence of malabsorption due to the enteropathy, but renal abnormalities are also observed, so it is as of yet unknown how and where this endothelial protein affects Mg^{2+}. Additional research is needed to decipher the molecular mechanism underlying hypomagnesemia in these syndromes.

Table 2. Mechanisms of drug-induced Mg^{2+} deficiencies

Mechanism	Drug	Estimated frequency*	Refs
TRPM6/7-mediated Mg^{2+} uptake	Cetuximab	20–30%	[18,91,92]
	Calcineurin inhibitors	10–50% (CsA) 50–98% (tacrolimus)	[97–101]
	Proton pump inhibitors	5–15%	[106–108]
Paracellular Mg^{2+} reabsorption in the TAL	Loop diuretics	Only with additional risk factors	[108,121–124]
	Aminoglycosides	30%	[127,128]
	Sirolimus	10%	[129,130]
Structural integrity of the DCT	Thiazide diuretics	10–15%	[126,134,135]
	Cisplatin	70–100%	[138–140]
	Amphotericin B	<30%	[143–145]
Mg^{2+} extrusion driven by the Na$^+$/K$^+$-ATPase	Digoxin	10–20%	[148–150]
Other mechanisms	β-adrenergic agonists	20–50%	[151–153]

TRPM6/7, transient receptor potential melastatin type 6/7; CsA, cyclosporine A; TAL, thick ascending limb of Henle's loop; DCT, distal convoluted tubule.
* Incidences of hypomagnesemia are often not studied in large populations. The frequencies are based on the provided references.

Drug-induced hypomagnesemia

Hypomagnesemia is a side effect of various drugs (Table 2). Interestingly, hypomagnesemia-causing drugs and hereditary causes of hypomagnesemia share common pathways, confirming the essential role of these pathways for Mg^{2+} (re)absorption in the intestine and kidney. In this section of the review, hypomagnesemia-inducing drugs are described in the context of the pathways described earlier, which provides various additional insights into these pathways.

Luminal Mg^{2+} uptake in the intestine and distal convoluted tubule

Somatic gain-of-function mutations in *EGFR* are associated with various cancers, which has led to the development of EGFR inhibitors for the treatment of lung, head and neck, colorectal and pancreas cancer[89]. The treatment options are either monoclonal antibodies, which bind EGFR extracellularly, or tyrosine kinase inhibitors (TKI), which bind intracellularly and prevent phosphorylation of the receptor[90]. In line with the role of EGF signalling in TRPM6 activation, hypomagnesemia is a common side effect of treatment with the EGFR antibody cetuximab, occurring in 20–30% of the patients[18,91,92]. Cetuximab prevents the EGF-induced activation of TRPM6 in kidney and colon cells, indicating the hypomagnesemia can be attributed to a decrease in both renal and intestinal (re)absorption[18,93]. Hypomagnesemia is not reported in patients treated with EGFR TKI[94]. However, EGFR TKI are less specific than EGFR antibodies and their anti-tumour effects often do not solely rely on the inhibition of EGFR, but also complementary pathways[90]. The specific inhibition of EGF signalling may therefore not be sufficient to induce hypomagnesemia in TKI treatment. Modest decreases in Mg^{2+} levels have been observed in animal models, however, indicating hypomagnesemia should not be completely ruled out in patients treated with EGFR TKI[95,96].

Another class of drugs that causes hypomagnesemia through interference with TRPM6 function are calcineurin inhibitors such as cyclosporine A (CsA) and tacrolimus, which are immunosuppressants used in transplant recipients. Hypomagnesemia is almost always reported in cohorts of calcineurin inhibitor-treated patients, with incidences ranging from 10 to 50% for CsA and from 50 up to 98% for tacrolimus[97–101]. CsA and tacrolimus were found to reduce the expression of TRPM6[102,103]. This effect may be mediated by the transcription factor c-Fos, as c-Fos is down-regulated by CsA and inhibition of c-Fos decreases both TRPM6 expression and Mg^{2+} uptake[102]. Moreover, renal EGF production is decreased in CsA-treated patients, indicating TRPM6 down-regulation may also occur through lowered EGF signalling in the DCT[104].

Proton pump inhibitors (PPI) are used for gastric-acid-related diseases such as peptic ulcers and are among the most-used drugs worldwide[105]. Hypomagnesemia is a side effect that occurs in 5–15% of PPI users and can become very severe, particularly after long-term treatment[106–108]. Oral supplementation of Mg^{2+} is often insufficient to fully restore normal Mg^{2+} levels in patients with PPI-induced hypomagnesemia[109]. Moreover, a reduced Mg^{2+} excretion is observed in these patients, indicating the kidney still functions normally and partially counteracts the hypomagnesemia by increasing Mg^{2+} reabsorption[110]. Therefore, it is generally accepted that PPI reduce the intestinal absorption of Mg^{2+}. Since a mild decrease in Mg^{2+} uptake in the intestine alone can usually be compensated, the risk of PPI-induced hypomagnesemia is strongest when additional factors contributing to Mg^{2+} depletion are present, such as the use of calcineurin inhibitors and diuretics, single nucleotide polymorphisms in *TRPM6* or increasing age[108,111–114]. PPI increase the pH of the gastrointestinal tract by inhibiting the release of gastric acid, which may explain the malabsorption since Mg^{2+} is more soluble and TRPM6 and TRPM7 show higher activity at lower pH values[12]. In a series of N-of-1 trials, lowering the luminal pH by supplementation of the naturally occurring polysaccharide inulin

indeed significantly improved the PPI-induced hypomagnesemia[115]. Since the decrease in pH after inulin treatment occurs because of fermentation by colonic bacteria[116], the microbiota might play an important role in Mg^{2+} (mal)absorption. Indeed, PPI alter the gut microbiota of patients[117–119] and a PPI-induced decrease in microbiota diversity is associated with hypomagnesemia in mice[120]. These data highlight the importance of the microbiota in generating a favourable environment for Mg^{2+} absorption in the intestine (Fig. 1a).

Paracellular Mg^{2+} reabsorption in the thick ascending limb of Henle's loop

Loop diuretics are a class of drugs that lower the blood pressure by inhibiting Na^+ reabsorption in the TAL, thereby promoting diuresis. The use of loop diuretics is associated with increased Mg^{2+} excretion[121–124]. Loop diuretics inhibit NKCC2 and therefore interfere with the lumen-positive voltage required for paracellular cation uptake in the TAL[38]. This inhibition particularly affects the paracellular transport of divalent cations such as Mg^{2+}[125]. Despite the decreased Mg^{2+} reabsorption in the TAL, some studies report no association between loop diuretics and hypomagnesemia while others only observe it in patients already prone to Mg^{2+} depletion due to heart failure or PPI use[108,123,124,126]. This indicates some additional risk factor has to be present before Mg^{2+} deficiency develops. Similar to the Mg^{2+} levels in patients with CASR mutations, whether hypomagnesemia develops likely also depends on the capacity of the DCT to compensate for the Mg^{2+} loss in the TAL[43].

Additionally, hypomagnesemia is observed in about 30 % of patients treated with aminoglycosides, a class of antibiotics used for tuberculosis and other bacterial infections[127,128]. Moreover, the mTOR inhibitor sirolimus (rapamycin), which is used for transplant recipients as an alternative to calcineurin inhibitors, leads to hypomagnesemia in about 10 % of cases[129,130]. Aminoglycosides and sirolimus decrease the expression of NKCC2, suggesting mechanistic similarities to loop diuretic-induced Mg^{2+} loss[131,132]. In addition, aminoglycosides are able to activate CaSR[133], which inhibits paracellular uptake of Ca^{2+} and Mg^{2+}. By affecting these two pathways simultaneously, it may be expected that aminoglycosides cause a rather severe wasting of Mg^{2+} in the TAL, which would explain the relatively high incidence compared to the loop diuretic- and sirolimus-induced hypomagnesemia.

Structural integrity of the distal convoluted tubule

Thiazide diuretics are among the most commonly used antihypertensive drugs and long-term use frequently leads to increased Mg^{2+} excretion, which is associated with a 2- to 3-fold increased risk of developing hypomagnesemia[126,134,135]. Thiazides promote diuresis by inhibiting NCC. Therefore, it can be expected that thiazide-induced Mg^{2+} loss shares many mechanistic similarities to the hypomagnesemia observed in Gitelman syndrome. Indeed, the DCT atrophy associated with

Gitelman syndrome is also seen in rats treated with thiazide[136,137]. However, other studies contradict this finding and report no deleterious effects on the DCT length in thiazide-treated mice[47]. This inconsistency may be explained by interspecies variation and/or differences in dosages. Interestingly, TRPM6 down-regulation still occurred in these thiazide-treated mice, indicating NCC regulates TRPM6 not just through DCT development but also via a more direct mechanism[45]. If a direct link between NCC and TRPM6 indeed exists, it still remains to be determined whether this mechanism or structural changes to the DCT underlie thiazide-induced hypomagnesemia, or whether it is a combination of the two.

Various drugs have nephrotoxic effects, which can lead to damage to the DCT and consequently disturbances in Mg^{2+} reabsorption. The majority of patients treated with the chemotherapeutic cisplatin, for example, develop hypomagnesemia if no measures are taken to prevent this[138–140]. Cisplatin accumulates in the kidney and causes nephrotoxicity that mainly affects tubular structures such as the DCT, resulting in disturbed reabsorption (Fig. 1c)[141]. Because of this widespread effect on the tubules, other electrolytes are also disturbed upon cisplatin treatment[142]. Similarly, the hypomagnesemia-inducing anti-fungal agent amphotericin B[143,144], as well as calcineurin inhibitors and aminoglycosides, can also cause nephrotoxicity[145–147]. In the case of calcineurin inhibitors and aminoglycosides, nephrotoxicity could thus be an additional explanation for the hypomagnesemia in addition to their respective effects on TRPM6 and NKCC2/CaSR. It should be noted that even though the DCT is prone to structural remodelling, the nephrotoxicity-induced changes affect multiple nephron segments, indicating the hypomagnesemia is not solely due to DCT damage.

Na^+-dependent extrusion of Mg^{2+} driven by the Na^+/K^+-ATPase

Hypomagnesemia is observed in 10–20 % of patients treated with digoxin, which increases renal Mg^{2+} excretion[148–150]. Digoxin inhibits the Na^+/K^+-ATPase and is used to treat arrhythmias and heart failure, since an increase in Na^+ in the cell leads to more intracellular Ca^{2+} and an increased contraction force. The digoxin-induced hypomagnesemia is in line with the importance of the Na^+/K^+-ATPase in basolateral Mg^{2+} extrusion in the kidney. Despite the ubiquitous function of the Na^+/K^+-ATPase in electrolyte transport, Mg^{2+} depletion upon digoxin treatment seems to be more frequent than disturbances of other electrolytes[149,150], again suggesting that Mg^{2+} homeostasis is relatively sensitive to decreased functioning of the Na^+/K^+-ATPase.

Others

β-adrenergic agonists activate the sympathetic nervous system and are frequently used in asthma patients to relax the airway muscles. Hypomagnesemia is observed in about 30 % of asthmatic patients and the use of β-adrenergic agonists contributes to this[151–153]. In this case, the mechanism is unrelated to the main pathways

of Mg^{2+} uptake. β-adrenergic agonists activate lipolysis, which results in an increased production of NEFA from triacylglycerols[154–156]. NEFA are able to bind Mg^{2+} and thereby decrease free Mg^{2+} in the blood, which could be interpreted as hypomagnesemia[157]. Indeed, the decrease in serum Mg^{2+} after treatment with β-adrenergic agonists coincides with an increase in NEFA levels[158]. This interaction between Mg^{2+} and NEFA is an important additional determinant of Mg^{2+} homeostasis that should not only be considered in β-adrenergic agonist treatment. For example, high levels of triacylglycerols, which correlates to high levels of NEFA, is also associated with hypomagnesemia in patients with type 2 diabetes[159]. Since NEFA affect free Mg^{2+} concentrations rather than the total Mg^{2+} content, it should be determined whether this has the same clinical effects as actual Mg^{2+} depletion.

Conclusions

Studies on hereditary causes of hypomagnesemia as well as hypomagnesemia-inducing drugs have been instrumental in the elucidation of the mechanisms involved in intestinal and renal Mg^{2+} (re)absorption (Fig. 1). Using this knowledge, novel treatment strategies and therapeutics can be developed to target these mechanisms and tackle the increasing problem of Mg^{2+} deficiency. For example, it has now become clear that serum Mg^{2+} levels can be increased not only by sufficient Mg^{2+} intake but also by healthy diets that sustain the gut microbiota and limit NEFA levels. A promising application of this is the use of dietary fibres that stimulate the microbiota and improve serum Mg^{2+} concentrations[115]. Development of additional treatment strategies is essential for patients with rare hereditary causes of hypomagnesemia and large patient groups dependent on hypomagnesemia-causing drugs.

Acknowledgements

Financial Support

Conflict of Interest

None.

Authorship

The authors had sole responsibility for all aspects of preparation of this paper.

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