Inherited disorders of renal hypomagnesaemia

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ABSTRACT

The kidney plays a key role in the maintenance of normal magnesium balance. The distal tubule of the kidney, namely the thick ascending limb of the loop of Henle and the distal convoluted tubule, is crucial for the regulation of serum magnesium levels and body magnesium content. The identification of molecular defects related to rare inherited magnesium losing disorders has contributed greatly to a better understanding of the process of renal magnesium handling. Since the number of genetic defects related to magnesium metabolism is still increasing, it might be expected that our knowledge on magnesium physiology will further improve. This knowledge will hopefully lead to therapeutic strategies that enable specific therapies for patients suffering from the symptoms and possible sequelae of chronic magnesium depletion.

Keywords: CLDN16, hypomagnesaemia, magnesium, renal genetics, TRPM6

INTRODUCTION

Magnesium has an essential role in many different biological activities (Table 1). A normal extracellular magnesium concentration is maintained by adaptive processes in response to quantitative changes of the intestinal uptake. In the gut, magnesium absorption is achieved in two different ways: (i) by simple diffusion through a paracellular pathway that is important at high intraluminal magnesium concentrations and (ii) by an active transcellular absorption mediated by the magnesium-permeable ion channel TRPM6, which is also active at low intraluminal concentrations.

The regulation of plasma magnesium concentration primarily resides in the kidney [1]. Approximately 70% of plasma magnesium is in the ionic form. The remaining magnesium is bound to circulating proteins or complexed with citrate, oxalate and phosphate ions. About 80% of the total plasma magnesium is filtered through the glomeruli and varies with the amount bound to non-filterable proteins. Of this amount, 15–20% is reabsorbed by the proximal tubule. The thick ascending limb (TAL) of Henle’s loop, mainly the cortical TAL, plays a major role in reclaiming filtered magnesium (55–70%). In this nephron segment, transepithelial magnesium reabsorption is passive through the paracellular pathway (Figure 1).

The driving force for magnesium (and also calcium) reabsorption is the positive luminal transepithelial voltage, which is generated by potassium recycling across the apical membrane. Any influence that alters this voltage will affect magnesium reabsorption in the TAL. In addition, the permeability of the paracellular pathway itself also plays an important role in determining magnesium transport. Paracellular magnesium movement is influenced by electrostatic charges of proteins within this route. Moreover, in the TAL, there appears to be selectivity of this pathway for divalent cations. Members of the claudin family of tight junction proteins (including Claudin-16 and Claudin-19) have been identified in the TAL and are involved in controlling magnesium and calcium permeability of the paracellular pathway [2, 3]. All members of the claudin protein family have four transmembrane domains, two extracellular loops and cytoplasmic amino- and carboxyl-terminal domains. The first extracellular loop consists of ~50 amino acids with intercalated negative and positive charges that contribute to paracellular ion selectivity through electrostatic effects. Claudins cis associate within the cell membrane as dimers or oligomers. These associations are followed by trans interactions between claudins in adjacent cells. Both types of interactions can involve either a single or different types of claudins. This allows a high compositional variability with different properties of the resulting tight junction strand (for review see [4]).

In the distal convoluted tubule (DCT), only 5–10% of the filtered magnesium is reabsorbed. It should be noted that the
DCT mediates the selective regulation of magnesium reabsorption and plays an important role in determining the final urinary excretion. Magnesium transport within the DCT is transcellular and active in nature (Figure 2). Magnesium enters the cell through selective ion channels (TRPM6) across the apical membrane, driven by the transmembrane negative electrical potential. Magnesium is actively extruded at the basolateral membrane, possibly by a sodium-dependent exchange mechanism that is still unresolved at the molecular level [1]. Only 3–5% of the filtered load normally appears in the urine.

Magnesium deficiency may occur as a result of reduced dietary intake, intestinal malabsorption or renal loss. Latent hypomagnesaemia is not a rare finding in either the general population (prevalence 2–13% [5, 6]) or in hospitalized patients (up to >50% in critically ill patients [7, 8]). The underlying disease conditions comprise acquired or hereditary disorders of magnesium handling, most of them due to renal magnesium loss. Acquired hypomagnesaemia is a well-known side effect of a number of different medications, such as thiazide diuretics, proton pump inhibitors, cisplatin, aminoglycoside antibiotics, calcineurin inhibitors or antibodies against epidermal growth factor receptor (EGF receptor) (overview in [9]). Hypomagnesaemia is also a common finding in the context of alcohol consumption, uncontrolled diabetes mellitus or acute pancreatitis. In comparison to acquired hypomagnesaemia, hereditary magnesium wasting disorders are relatively rare but for our understanding of renal magnesium handling, these rare disease entities have proved to be extremely helpful. The careful clinical characterization of affected individuals, together with experimental genetic studies, has allowed the identification of a number of genes involved in the pathophysiology of these disorders and provided insight into epithelial magnesium transport at the molecular level. This review addresses the current understanding of inherited hypomagnesaemic disorders.
meters in serum and urine, together with extrarenal single families. The assessment of additional biochemical para-absorption in the DCT, to rare disorders discovered only in Gitelman syndrome (GS) with a primary defect in NaCl re-absorption in the TAL. Most likely, the inhibition of calcium and magnesium reabsorption in the kidney. However, 20 years after its first description, the exact role of the CaSR is still not fully understood. It is now accepted that changes in extracellular calcium concentration influence the transport of NaCl, water, calcium and magnesium by the renal tubule. The traditional hypothesis holds that the CaSR is centrally involved in all adaptive responses to changes in extracellular calcium. This hypothesis has been challenged by experimental evidence that the renal CaSR specifically controls tubular calcium and magnesium transport in the TAL, independent of PTH, whereas it does not significantly affect the reabsorption of NaCl [12] (for review see [13]).

Activating mutations of the CASR gene were first identified in autosomal dominant hypocalcaemia (ADH). Affected individuals present with hypocalcaemia, inadequately low PTH levels and increased fractional calcium excretion rates. More than 50% of the patients also have significant hypomagnesaemia [14, 15]. Carpopedal spasms and/or seizures are typical symptoms, but ADH may also be asymptomatic. The differentiation from primary hypoparathyroidism is important for ADH patients because treatment with vitamin D can result in overt hypercalciuria, with the development of nephrocalcinosis and impairment of renal function. Therefore, therapy of hypocalcaemia in ADH with vitamin D and calcium should be restricted to symptomatic patients.

Activating CaSR mutations shift the set point of the receptor to a level of enhanced sensitivity for extracellular calcium and magnesium. This results in a decrease of PTH secretion and an inhibition of calcium and magnesium reabsorption in the TAL. Most likely, the inhibition of calcium and magnesium reabsorption in the TAL is due to a selective reduction of

**Table 2. Inherited disorders of renal magnesium loss**

<table>
<thead>
<tr>
<th>Disorder</th>
<th>OMIM</th>
<th>Inheritance</th>
<th>Gene</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary NaCl-wasting disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classic Bartter syndrome</td>
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<td>AR</td>
<td>CLCNKB</td>
<td>CIC-Kb, chloride channel</td>
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<tr>
<td>Autosomal dominant hypocalcaemia</td>
<td>601199</td>
<td>AD</td>
<td>CASR</td>
<td>CaSR, calcium/magnesium-sensing receptor</td>
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<td>AR</td>
<td>SLC12A3</td>
<td>NCCT, NaCl co-transporter</td>
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<td>AR</td>
<td>KCN10</td>
<td>Kir4.1, potassium channel</td>
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<tr>
<td>Familial hypomagnesaemia with hypercalciuria/nephrocalcinosis</td>
<td>248250</td>
<td>AR</td>
<td>CLDN16</td>
<td>Claudin-16, tight junction</td>
</tr>
<tr>
<td>Hypomagnesaemia/secondary hypocalcaemia</td>
<td>248190</td>
<td>AR</td>
<td>CLDN19</td>
<td>Claudin-19, tight junction</td>
</tr>
<tr>
<td>Isolated dominant hypomagnesaemia</td>
<td>602014</td>
<td>AR</td>
<td>TRPM6</td>
<td>TRP6, cation channel</td>
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<tr>
<td>Isolated recessive hypomagnesaemia</td>
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<td>AD</td>
<td>FXYD2</td>
<td>Gamma subunit Na/K/ATPase</td>
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<tr>
<td>Transient neonatal hyperphenylalaninemia</td>
<td>176260</td>
<td>AD</td>
<td>KCNA1</td>
<td>Kv1.1, potassium channel</td>
</tr>
<tr>
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<td>AR</td>
<td>CNNM2</td>
<td>Cyclin M2</td>
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<tr>
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<td>AR</td>
<td>PCBD1</td>
<td>PCBD1, tetrahydrobipterin metabolism</td>
</tr>
</tbody>
</table>
| Autosomal dominant hypocalcaemia (CaSR). The extracellular calcium/magnesium-sensing receptor (CaSR) is critical for calcium and magnesium homeostasis by influencing not only parathyroid hormone (PTH) secretion but also regulating calcium and magnesium reabsorption in the kidney. However, 20 years after its first description, the exact role of the CaSR is still not fully understood. It is now accepted that changes in extracellular calcium concentration influence the transport of NaCl, water, calcium and magnesium by the renal tubule. The traditional hypothesis holds that the CaSR is centrally involved in all adaptive responses to changes in extracellular calcium. This hypothesis has been challenged by experimental evidence that the renal CaSR specifically controls tubular calcium and magnesium transport in the TAL, independent of PTH, whereas it does not significantly affect the reabsorption of NaCl [12] (for review see [13]).

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**INHERITED RENAL HYPMAGNESAEAMIA**

Hereditary hypomagnesaemia comprises a still growing number of rare genetically determined disorders, either primarily or secondarily affecting renal magnesium handling. In recent years, numerous genetic defects in components of renal tubular electrolyte transport or regulating factors have been described (Table 2). The spectrum ranges from the most frequent variant, Gitelman syndrome (GS) with a primary defect in NaCl re-absorption in the DCT, to rare disorders discovered only in single families. The assessment of additional biochemical parameters in serum and urine, together with extrarenal findings and the mode of inheritance, may help to confine the possibly underlying genetic defects (Table 3).

Hypomagnesaemia associated with hypokalaemic salt-wasting disorders

The reabsorption of NaCl along the nephron affects the membrane potential of tubular epithelia and is involved in the generation of the transepithelial potential, which are both a prerequisite for magnesium reabsorption. Primary salt-wasting disorders with secondary hypokalaemia and metabolic alkalosis, also known as Bartter-like syndromes, impair tubular reabsorption of NaCl in different parts of the distal nephron. The renal conservation of magnesium is secondarily affected to a varying extent, according to the affected nephron segment.

**Classic Bartter syndrome.** Classic Bartter syndrome (cBS) is an autosomal recessive disease. The clinical symptoms of cBS vary widely, but the majority of patients develop hypokalaemia, hypochloremic alkalosis and failure to thrive during the first year of life, as described in Bartter’s initial report [10]. The hallmarks of antenatal BS such as polyhydramnios, prematurity, massive loss of NaCl and water from birth and nephrocalcinosis are usually absent, which allows the differentiation of cBS from restricted to symptomatic patients.

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**FULL REVIEW**

Inherited hypomagnesaemia
the paracellular permeability [13]. For magnesium, the inhibition of PTH-stimulated reabsorption in the DCT may further increase the observed renal magnesium loss [16].

In rare cases, heterozygous mutations in CaSR activate the receptor to the extent that they are fully activated under normal serum calcium concentrations. In such patients, a typical Bartter-like phenotype with pronounced renal loss of NaCl and metabolic alkalosis has been described during follow-up [16, 17]. The exact mechanism leading to this renal loss of NaCl is not easily explained because it has been shown that NaCl reabsorption in the TAL, the main site of expression in the kidney, is not influenced by activation of the CaSR [13]. Further studies will be needed to clarify the underlying pathophysiology in more detail.

**Gitelman syndrome.** GS is the most frequent inherited salt-wasting disorder, with an estimated prevalence of ~1:40 000. GS is characterized by hypokalaemic alkalosis, hypomagnesaemia and hypocalciuria [18, 19]. Patients usually present during late childhood or adolescence with symptoms of muscle weakness or overt tetanies related to hypomagnesaemia. Parasthesia and chronic fatigue are also frequent findings. Notably, some patients with GS may present at very young ages with symptomatic electrolyte disturbances and failure to thrive [20]. After long-lasting hypomagnesaemia, older patients may suffer from chondrocalcinosis. The aetiology of calcium pyrophosphate dehydrate crystal deposition (chondrocalcinosis) is very likely linked to chronic hypomagnesaemia in GS, because magnesium is a cofactor of various pyrophosphatases including the alkaline phosphatase [21]. However, many so-called ‘asymptomatic’ patients have also been reported, and GS is often diagnosed after the assessment of serum electrolytes for other reasons. Nevertheless, it has been demonstrated that GS cannot be considered a mild disorder, because none of the studied patients were truly asymptomatic. Salt craving, nocturia and paraesthesia are the most frequent symptoms of GS, and significantly influence the overall reduced quality of life [22].

GS is caused by recessive mutations in the SLC12A3 gene encoding the thiazide-sensitive chloride cotransporter NCCT, which is exclusively expressed in the apical membrane of DCT cells [23, 24]. These mutations lead to a disruption of NaCl reabsorption in the DCT, which results in mild volume contraction and subsequent hyperaldosteronism with hypokalaemic alkalosis. Passive calcium reabsorption in the proximal tubule and reduced expression of TRPM6 in the DCT could explain the pathogenesis of hypocalciuria and hypomagnesaemia as seen in most GS patients [25].

**EAST/SeSAME syndrome.** In 2009, a newly characterized clinical syndrome with autosomal recessive inheritance combining epilepsy, ataxia, sensorineural deafness and renal NaCl wasting with/without mental retardation was described under the acronyms EAST or SeSAME syndrome [26, 27]. During infancy, the neurological symptoms dominate the clinical picture, whereas the renal loss of NaCl is often recognized only later during the course of the disease [28]. The renal phenotype includes hypokalaemic alkalosis, hypomagnesaemia and hypocalciuria.

EAST/SeSAME syndrome is caused by loss-of-function mutations in KCNJ10, which encodes the potassium channel Kir4.1 [26, 27]. The expression pattern of Kir4.1 fits to the disease phenotype with highest expression in brain, the stria vascularis of the inner ear, and in the distal nephron, especially in the DCT. Kir4.1 is involved in potassium recycling over the basolateral membrane and thereby maintaining the function of the Na-K-ATPase. Loss of Kir4.1 function leads to a depolarization of the basolateral membrane and to a reduction of the driving force for basolateral anion channels as well as sodium-coupled exchangers [29]. By this mechanism, KCNJ10 defects could also affect the putative sodium/magnesium exchanger and possibly explain the magnesium wasting observed in EAST/SeSAME syndrome.

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**Table 3. Clinical and biochemical characteristics in inherited magnesium losing disorders**

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Serum Mg&lt;sup&gt;2+&lt;/sup&gt;</th>
<th>Serum Ca&lt;sup&gt;2+&lt;/sup&gt;</th>
<th>Serum K&lt;sup&gt;+&lt;/sup&gt;</th>
<th>Blood pH</th>
<th>Urine Mg&lt;sup&gt;2+&lt;/sup&gt;</th>
<th>Urine Ca&lt;sup&gt;2+&lt;/sup&gt;</th>
<th>Other findings</th>
</tr>
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<tr>
<td>Classic Bartter syndrome</td>
<td>↓↓</td>
<td>↑↑</td>
<td>↓↓</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↓↓</td>
<td>Failure to thrive, polyuria</td>
</tr>
<tr>
<td>Autosomal dominant</td>
<td>↓</td>
<td>↓</td>
<td>↓↓</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑↑</td>
<td>Hypoparathyroidism</td>
</tr>
<tr>
<td>Gitelman syndrome</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>Chondrocalcinosis</td>
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<td>EAST/SeSAME syndrome</td>
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<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>Epilepsy, ataxia, deafness</td>
</tr>
<tr>
<td>Familial hypomagnesaemia with hypercalciuria/nephrocalcinosis</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
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<td>↓</td>
<td>↓</td>
<td>Nephrocalcinosis, renal failure</td>
</tr>
<tr>
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<td>↓</td>
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<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>Ocular abnormalities</td>
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<td>↓</td>
<td>↓</td>
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<td>↓</td>
<td>↓</td>
<td>None</td>
</tr>
<tr>
<td>Related to KCNA1 defects</td>
<td>↓</td>
<td>↓</td>
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<td>↑</td>
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<td>↑</td>
<td>Episodic ataxia, myokymia</td>
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<td>Related to CNNM2 defects</td>
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<td>↑</td>
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</tr>
<tr>
<td>HNF1B nephropathy</td>
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<td>↓</td>
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<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>Renal anomalies, interstitial nephritis, diabetes (MODY3)</td>
</tr>
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<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>Diabetes (MODY)</td>
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<tr>
<td>Isolated recessive hypomagnesaemia</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>Mental retardation</td>
</tr>
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</table>
Familial hypomagnesaemia with hypercalciuria and nephrocalcinosis

Familial hypomagnesaemia with hypercalciuria and nephrocalcinosis (FHHNC) is an autosomal recessive tubular disease. Since its first description, >100 patients have been reported, allowing a comprehensive characterization of the clinical spectrum of this disorder [30–33]. Almost all affected individuals develop the characteristic triad of hypomagnesaemia, hypercalciuria and nephrocalcinosis. In addition, the majority of patients present during early childhood with recurrent urinary tract infections, polyuria/polydipsia, nephrolithiasis and/or failure to thrive. Clinical signs of severe hypomagnesaemia such as seizures and muscular tetany are less common. Additional biochemical abnormalities include elevated PTH levels before the onset of chronic renal failure, hypocitraturia and hyperuricemia. In a subset of patients, ocular involvement including severe myopia, nystagmus or macular coloboma has been described [3, 30, 32, 33]. The prognosis of FHHNC patients is rather poor with a high risk of progressive renal failure. The degree of renal calcification has been correlated with progression of chronic renal failure [30]. Therefore, in addition to oral magnesium supplementation, current therapy aims to reduce calcium excretion in order to prevent the progression of nephrocalcinosis and stone formation. However, these therapeutic strategies do not seem to significantly halt the progression of renal failure [30, 31].

Based on clinical observations and clearance studies, it has been postulated that the primary defect in FHHNC was related to abnormal magnesium and calcium reabsorption in the TAL. In 1999, Simon et al. [2] identified a new gene (CLDN16, formerly PCLN1), which is mutated in patients with FHHNC. CLDN16 codes for Claudin-16, a member of the claudin family that is important for the formation and function of tight junctions. The individual composition of tight junction strands with different Claudins confers the characteristic properties of different epithelia regarding paracellular permeability and/or transepithelial resistance.

The majority of CLDN16 mutations reported in FHHNC are simple missense mutations affecting the transmembrane domains and the extracellular loops, with a particular clustering in the first extracellular loop that contains the ion selectivity filter. Within this domain, patients originating from Germany or Eastern European countries exhibit a common mutation (L151F) due to a founder effect [31]. Defects in CLDN16 have also been shown to underlie the development of a chronic interstitial nephritis in Japanese cattle that rapidly develop chronic renal failure shortly after birth [34]. Interestingly, affected animals show hypocalcaemia but no hypomagnesaemia, which might be explained by advanced renal failure present at the time of examination. In contrast to the point mutations identified in human FHHNC, large deletions of CLDN16 are responsible for the disease in cattle, which might explain the more severe phenotype with early-onset renal failure. However, Cldn16 knockout mice do not display renal failure during the first months of life [35]. In FHHNC patients, progressive renal failure is generally thought to more likely be a consequence of massive urinary calcium wasting and nephrocalcinosis. A study of a large cohort of FHHNC patients showed that the presence of CLDN16 mutations leading to a complete loss of function of both alleles display a younger age at manifestation as well as a more rapid decline in renal function, compared with patients with at least one allele with residual Claudin-16 function [36]. These findings support the theory that a complete lack of Claudin-16 is associated with a more severe phenotype, whereas a residual function delays the progression of renal failure.

Molecular genetic studies in FHHNC patients with a severe ocular involvement led to the identification of mutations in a second member of the claudin family, Claudin-19 (encoded by CLDN19) [3]. Claudin-19 is expressed together with Claudin-16 predominantly in the TAL. Tight-junction strands in this part of the renal tubule also express Claudin-10 and Claudin-18. Although Claudin-10 and Claudin-18 can maintain the barrier function of the tight junction complex in the absence of Claudin-16 and -19, these tight junctions displayed a loss in cation permeability. It remains unknown, whether Claudin-16 and -19 directly assemble the paracellular pore structure selective for magnesium and calcium or are simply involved in generating the cation selectivity of the tight junction complex, which is required for maintaining the lumen-positive potential in the TAL [37]. In this context, it is noteworthy that Claudin-16 and Claudin-19-deficient mice also display increased renal losses of sodium and potassium in addition to the disturbance in renal magnesium and calcium handling [38].

Hypomagnesaemia with secondary hypocalcaemia

Hypomagnesaemia with secondary hypocalcaemia (HSH) is an autosomal recessive disorder caused by mutations in the TRPM6 gene coding for TRPM6 [39, 40]. Since its first description in 1968, at least 50 HSH kindreds have been described [40–42]. Most patients present in early infancy with generalized seizures that are refractory to anticonvulsant treatment. Muscle spasms or tetany may also occur. Laboratory evaluation at initial presentation reveals very low magnesium levels of ~0.2 mmol/L. Hypomagnesaemia is accompanied by hypoparathyroidism and consecutive hypocalcaemia. Hypoparathyroidism is thought to result from an inhibition of PTH synthesis and secretion in the presence of extreme hypomagnesaemia. In addition, PTH-induced release of calcium from bone is substantially impaired in hypomagnesaemia. This hypocalcaemia is resistant to treatment with calcium or vitamin D.

Acute treatment of HSH consists of immediate administration of intravenous magnesium, which rapidly leads to relief of clinical symptoms, normocalcaemia and normalization of PTH levels. Acute parenteral therapy is followed by lifelong high-dose oral magnesium supplementation. Delay in diagnosis may lead to neurological deficits or may even be fatal, as seizures are refractory to anticonvulsant treatment. Several HSH patients with severe mental retardation after long-lasting seizures have been reported.

In contrast to all other known forms of hereditary hypomagnesaemia, pathophysiologic studies in affected individuals pointed to a primary defect in intestinal magnesium absorption. The presence of an additional renal magnesium loss in HSH was controversial until magnesium-loading studies clearly demonstrated a renal magnesium leak. With rising serum magnesium...
levels during substitution, renal magnesium loss, which is barely detectable at initial presentation, becomes evident and demonstrates a decreased renal threshold for magnesium [40].

A positional candidate gene approach enabled the identification of mutations in the TRPM6 gene as the underlying defect in HSH [39, 40]. TRPM6 codes for a member of the transient receptor potential (TRP) family of cation channels. The TRPM subfamily comprises eight members that exhibit a significant diversity in domain structure as well as cation selectivity and activation mechanisms. TRPM6 and TRPM7 are distinct from all other known ion channels because they harbour a protein kinase domain in their respective carboxyl-termini and thus (together with TRPM2) represent prototypes of an intriguing new family of enzyme-coupled ion channels [43].

TRPM6 is highly homologous to TRPM7. The functional characterization of TRPM7 demonstrated permeability for various cations, including calcium and magnesium. Channel gating was shown to be regulated by intracellular magnesium and magnesium-nucleotide complexes [44]. Targeted deletion of TRPM7 in cell lines results in intracellular magnesium depletion and growth arrest [45]. To date, only one group has succeeded in the functional expression of TRPM6 in a mammalian cell line [46]. The authors reported channel properties similar to those observed for TRPM7. In contrast, another group demonstrated that heteromultimerization with TRPM7 is essential for correct membrane targeting of TRPM6 [47]. The detection of TRPM6 expression in the DCT, together with the functional studies in HSH patients that clearly demonstrated a renal magnesium leak, points to an important role of TRPM6 for active transcellular magnesium reabsorption in the DCT [39, 46]. Whether TRPM6 alone or in cooperation with TRPM7 constitutes the apical magnesium channel in DCT cells remains to be clarified in future studies.

Thus far, most mutations described in HSH result in truncated TRPM6 proteins, either by nonsense, frame shift or splice site mutations [39, 40]. Only a few missense mutations of TRPM6 have been identified [39, 42, 48]. Functional characterization of these mutations revealed a complete loss of function of the TRPM6 protein [42, 47, 48]. It is intriguing to speculate whether subtle changes in TRPM6 function by single point mutations might result in a less severe clinical picture or even in subclinical magnesium deficiency. In this context, it is interesting to note that two single nucleotide polymorphisms in TRPM6 have been identified as a risk factor for the development of an impaired glucose metabolism during pregnancy or at older ages [49, 50]. There is experimental evidence that insulin stimulates TRPM6 activity by increasing its expression at the cell surface. This activation is abrogated in the presence of these two variants, namely V1393I and K1584E [50].

The observation in HSH patients that the administration of high oral doses of magnesium is successful in achieving at least subnormal serum magnesium levels supports the existing evidence of two independent transport systems for magnesium in the gastrointestinal tract. TRPM6 probably represents a molecular component of active transcellular magnesium transport in the small intestine [39, 46]. An increased intraluminal magnesium concentration achieved by increased oral intake could compensate for the defect of the active transcellular pathway by increasing absorption via the passive paracellular route.

**Isolated dominant hypomagnesaemia**

Isolated dominant hypomagnesaemia (IDH) represents a heterogeneous group of disorders, that was first described by Geven et al. in 1987 [51]. To date, three different genetic causes have been characterized that all affect magnesium reabsorption in the DCT.

**FXYD2.** IDH was first linked to a single missense mutation in the FXYD2 gene, which codes for the γ-subunit of the Na-K-ATPase. Only two related families with the same FXYD2 mutation have been described thus far [51, 52]. The index patients of both families presented with generalized seizures at a young age. Other mutation carriers from both families remained asymptomatic except for the development of chondrocalcinosis at an adult age. A 24Mg-retention study in one index patient pointed to a primary renal defect, although intestinal magnesium was preserved [51]. In addition, urinary calcium excretion rates were found to be low in all hypomagnesaemic family members.

The γ-subunit (isoform γ-b) is expressed in the distal nephron, especially in the basolateral membrane of epithelial cells of the DCT. It represents a tissue-specific regulator of the Na-K-ATPase, which maintains the membrane potential and the sodium gradient.

Expression studies of the mutant G41R-γ-subunit revealed a dominant negative effect, leading to retention of the γ-subunit within the cell [53]. This likely dominant negative effect is strengthened by the observation that individuals with a large heterozygous deletion of chromosome 11q (including the FXYD2 gene) exhibit normal serum magnesium levels. Furthermore, it could be shown that wild-type γ-subunits oligomerize within the cell before trafficking to the plasma membrane. Since the G41R-mutant was shown not only to oligomerize with itself but also with wild-type subunits, the latter are also prevented from proper routing to the plasma membrane and incorporation into functional ATPase complexes [54]. The idea of a dominant negative effect is further substantiated by the finding that Fxyd2 knockout mice have no abnormalities in their magnesium metabolism [55]. However, the exact molecular mechanism fully explaining the disease phenotype remains to be further elucidated.

**KCNA1.** Genetic heterogeneity in IDH was demonstrated by the identification of a dominant-negative missense mutation in KCNA1 encoding the voltage-gated potassium channel Kv1.1 in a large Brazilian family [56]. The clinical phenotype includes muscle cramps, tetanic episodes, tremor and muscle weakness starting in infancy. Laboratory analyses revealed a renal magnesium leak without alterations in renal calcium handling.

Interestingly, KCNA1 mutations had also been identified in patients with episodic ataxia with myokymia (OMIM 160120), a neurologic disorder characterized by a periodic appearance of incoordination and imbalance as well as myokymia, an involuntary, spontaneous and localized trembling of muscles. In addition to muscle cramps and tetany attributed to
magnesium deficiency, these symptoms were also present in members of the aforementioned Brazilian kindred with hypomagnesaemia.

Functional characterization of the mutation revealed that coexpression of the mutant N255D-Kv1.1 with wild-type channel subunits resulted in a significant reduction in current amplitudes, indicating a dominant-negative effect. This effect likely stems from an impaired gating of the potassium channel tetramer, whereas trafficking to the plasma membrane is preserved [57].

Kv1.1 is colocalized with TRPM6 in the apical membrane of the DCT. Glaudemans et al. [56] proposed a model in which Kv1.1 mediates hyperpolarization of the DCT apical cell membrane potential as a prerequisite for TRPM6-mediated magnesium entry (Figure 2). These authors linked magnesium reabsorption in the DCT to potassium secretion and thus identified a new dependency between renal magnesium and potassium handling at the molecular level.

CNNM2. Another form of IDH has been recently linked to mutations in CNNM2 [58]. Previously, common variants in CNNM2 had been described to be associated with serum magnesium levels in a genome-wide association study [59]. Stuiver et al. [58] identified heterozygous CNNM2 mutations in two families with IDH. Clinical symptoms and age at manifestation were variable with symptoms ranging from seizures in early childhood to muscle weakness, vertigo and headache during adolescence. Other heterozygous carriers from both families remained asymptomatic. Except magnesium, serum electrolytes were normal. Thus far, it remains unclear if the finding of hypocalciuria seen in a number of other inherited magnesium wasting disorders (see above) is also a feature in patients with CNNM2 mutations.

The CNNM2 gene codes for CNNM2 or Cyclin M2, a transmembrane protein that is expressed in kidney at the basolateral membrane of TAL and DCT, but also in other organs, especially the brain [58]. Whereas a truncating frame-shift mutation was identified in one of the described families, affected individuals of the second family carried a missense mutation leading to a non-conservative amino acid exchange in CNNM2 [58]. Functional characterization of the mutant T568I-CNNM2 demonstrated that protein trafficking was preserved in HEK293 cells; however, patch clamp analyses revealed a significant reduction in magnesium-sensitive, inwardly-rectifying sodium currents [58].

HNF1B nephropathy

Hepatocyte nuclear factor 1β (HNF1B) is a transcription factor critical for the development of the kidney and pancreas. Heterozygous mutations in HNF1B were first implicated in a subtype of maturity-onset diabetes of the young (MODY5) before an association with developmental renal disease was reported. The renal phenotype is highly variable, including enlarged hyperechogenic kidneys, multicystic kidney disease, renal agenesis, renal hypoplasia, cystic dysplasia, as well as hyperuricemic nephropathy. The association with both symptom complexes led to the term renal cysts and diabetes syndrome. Recent data showed that this denomination may be misleading because the renal cystic phenotype and diabetes are not a constant clinical finding [60]. For this reason, the term HNF1B nephropathy has been introduced. HNF1B mutations are present in a heterozygous state, either inherited or de novo, and comprise point mutations as well as whole-gene deletions [61]. Interestingly, ~50% of affected individuals present with hypomagnesaemia of renal origin [61, 62]. The defect in renal magnesium conservation is accompanied by hypocalciuria. The HNF1B gene encodes a transcription factor regulating the expression of numerous renal genes including the FXYD2 gene, which contains several HNF1B-binding sites in its promoter region [62]. In accordance with the phenotype and in silico data, Adalat and colleagues showed that HNF1B could induce the expression of FXYD2 in vitro. Therefore, defective FXYD2 transcription represents a putative mechanism explaining renal magnesium wasting in patients with HNF1B mutations.

Transient neonatal hyperphenylalaninemia

Recently, renal magnesium loss has also been demonstrated in patients affected from transient neonatal hyperphenylalaninemia due to recessive mutations in PCBD1. These patients were shown to develop hypomagnesaemia during follow-up. MODY type diabetes was also observed in two of them. Functional studies revealed that PCBD1 is an essential dimerization cofactor of HNF1B. It was further demonstrated that the dimerization of PCBD1 with HNF1B stimulates the promoter activity of FXYD2 in the DCT, and that this stimulation is abrogated by mutations found in PCBD1 [63].

Isolated recessive hypomagnesaemia

Geven et al. [64] initially reported a form of isolated hypomagnesaemia in a consanguineous family indicating autosomal recessive inheritance. Two affected girls presented with generalized convulsions during infancy and neurodevelopmental deficits during follow-up. A 24 Mg-retention study in one patient pointed to a primary renal defect, while intestinal magnesium uptake was preserved [64]. Calcium excretion rates were in the normal range.

Using homozygosity mapping with subsequent screening of candidate genes, Groenestege et al. [65] identified a homozygous missense mutation in the EGF gene leading to a non-conservative amino acid exchange in the encoded pro-EGF protein (pro-epidermal growth factor) in the two sisters. Pro-EGF is a small peptide hormone expressed in various tissues including the kidney (predominantly in the DCT). Pro-EGF is a membrane protein that is inserted in both the luminal and basolateral membrane of polarized epithelia. After membrane insertion, it is processed into active EGF peptide. EGF activates specialized EGF receptors (EGFRs) that are expressed in the basolateral membrane. This activation leads to an increase in TRPM6 trafficking to the luminal membrane and increased magnesium reabsorption [66]. The mutation described in IRH (P1070L) disrupts the basolateral sorting motif in pro-EGF [65]. Therefore, the activation of EGFRs in the basolateral membrane is compromised, which ultimately leads to reduced magnesium reabsorption. Despite acting in a paracrine
fashion in the DCT, the authors speculate a role for EGF as a selectively acting magnesiotropic hormone [65].

CONCLUSIONS

During the last 15 years, the aetiologic and pathophysiology of numerous monogenic disorders leading to renal magnesium loss have been elucidated. Because many hereditary hypomagnesaemic disease cases remain unexplained, it is expected that more genetic defects related to renal magnesium handling will be identified. This should help to better characterize distinct disease phenotypes and to predict the course of the disease as well as improve our understanding of renal magnesium physiology. From a clinical perspective, this increased knowledge is eagerly awaited because of the therapeutic challenges that still remain, e.g. the prevention of chondrocalcinosis or the reduction of the common gastrointestinal side effects associated with magnesium supplementation. It remains to be seen, whether such specific therapeutic approaches in these rare inherited disorders will be useful also for acquired hypomagnesaemia, which is by far more frequent and therefore even more important for daily clinical practice.

CONFLICT OF INTEREST STATEMENT

None declared.

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