Recent Advances in Molecular Genetics of Hereditary Magnesium-Losing Disorders

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Abstract. Recent advances in molecular genetics in hereditary hypomagnesemia substantiated the role of a variety of genes and their encoded proteins in human magnesium transport mechanisms. This knowledge on underlying genetic defects helps to distinguish different clinical subtypes and gives first insight into molecular components involved in magnesium transport. By mutation analysis and functional protein studies, novel pathophysiologic aspects were elucidated. For some of these disorders, transgenic animal models were generated to study genotype-phenotype relations and disease pathology. This review will discuss genetic and clinical aspects of familial disorders associated with magnesium wasting and focuses on the recent progress that has been made in molecular genetics. Besides isolated renal forms of hereditary hypomagnesemia, the following disorders will also be presented: familial hypomagnesemia with hypercalcemia and nephrocalcinosis, hypomagnesemia with secondary hypocalcemia, Ca\(^{2+}/Mg\(^{2+}\)-sensing receptor–associated disorders, and disorders associated with renal salt-wasting and hypokalemic metabolic alkalosis, comprising the Gitelman syndrome and the Bartter-like syndromes.

Magnesium is the second most abundant intracellular cation and plays an important role for protein synthesis, nucleic acid stability, neuromuscular excitability, and oxidative phosphorylation. Under normal conditions, extracellular magnesium concentration is maintained at nearly constant values. Hypomagnesemia results from decreased dietary intake, intestinal malabsorption, or renal loss.

During the last four decades, numerous reports concerning inherited magnesium-losing disorders have been published and their distinctive phenotypic features have been intensively discussed. Whereas intestinal magnesium absorption is still poorly understood, phenotypic characterization of clinically affected patients and experimental studies of appropriate animal models have contributed to a growing knowledge of renal magnesium transport mechanisms. The identification of the most likely affected nephron segments in the kidney, the presentation of different modes of inheritance and the observation of additional characteristic symptoms promoted a classification into different subtypes of inherited magnesium-losing disorders.

In general, primary magnesium-wasting disorders are relatively rare. The prevalence of the more frequent entities, for example Gitelman and Bartter syndrome, has been estimated to be only approximately 1:50,000 (1). For most of the other disease entities, relatively few cases have been reported in the literature. Depending on the genotype, the clinical course is sometimes mild or even asymptomatic. Therefore, the disease prevalence might be underestimated for some of these syndromes. Several reports have demonstrated latent hypomagnesemia in a high percentage in the general population (2), e.g., 14.5% in a recent German study (3). Furthermore, associations of hypomagnesemia with common chronic diseases have been reported (4,5). Hypomagnesemia is frequently detected in patients with diabetes mellitus type II, arterial hypertension, coronary heart disease, or asthma bronchiale, and diminished magnesium is commonly related to an aggravation of these diseases. Hypomagnesemia predisposes to hyperglycemia in diabetes mellitus type II, and magnesium supplementation seems to retard the induction of insulin resistance (6). Reports have linked hypomagnesemia to mortality in coronary heart disease, and administration of magnesium has a cardioprotective effect in patients with acute myocardial infarction (7). The BP in patients with arterial hypertension can be lowered by magnesium supplementation (7). Magnesium treatment of subjects with chronic asthma is currently receiving attention, because of a role for magnesium in relaxation of the arterial and bronchial smooth muscle cells (8). This obvious coherence has provoked further interest in understanding magnesium transport mechanisms and elucidating possible roles of inherited predispositions to hypomagnesemia. The question arises whether genetic variations of genes underlying hereditary hypomagnesemia might contribute to the pathophysiology of these widespread chronic diseases.

Magnesium transport has been intensively studied in humans and various animal models, leading to accepted concepts underlying the pathophysiology of the different forms of hypomagnesemia (9). However, the electrophysiologic characterization of magnesium pathways has been complicated by...
unintentional simultaneous measurement of other cations so that the molecular correlates mediating mammalian magnesium transport components remained undefined. The first magnesium transporter genes have been cloned in bacteria and plants (10,11). Meanwhile, a human homologue of the yeast MRS2 gene, characterized as a mitochondrial magnesium transporter has been identified (12). Its relevance for magnesium homeostasis outside mitochondria remains to be determined, but it may be a candidate for genetic disturbances in cellular magnesium homeostasis.

A different approach to study components of magnesium transport arises from genetic analysis of families affected with magnesium-wasting diseases. Linkage disequilibrium studies on affected kindreds have enabled the chromosomal localization of several genes involved in hereditary hypomagnesemia; in the last decade, a number of genes have been identified by positional cloning. These genes have provided first insight into mammalian magnesium transport molecules. A detailed analysis of hereditary magnesium-losing disorders was given by Cole and Quamme (13) in this Journal only three years ago. Remarkable progress has been made in this field since that time. In this review, we would like to focus on the most recent research as it adds considerably to our understanding of renal magnesium conservation.

Physiology of Magnesium Homeostasis

In healthy humans, serum magnesium concentration is maintained in a narrow range of 0.7 to 1.1 mmol/L. This extracellular magnesium is in continuous exchange with magnesium stores in bone and muscle tissue. A balanced Western diet offers approximately 12 mmol (400 mg) magnesium per day; 6 mmol is absorbed, and 2 mmol is secreted in the intestine with a net gain of 4 mmol that is excreted in the urine. Diminished magnesium intake is balanced by enhanced magnesium absorption in the intestine and reduced renal excretion. These transport processes are regulated by metabolic and hormonal influences (9). A magnesium deficit is frequently associated with other electrolyte disturbances, including hypokalemia, hypophosphatemia, and hypocalcemia, adding to the presenting symptoms. Prolonged insufficiency of magnesium supply may result in the manifestation of anorexia, nausea, vomiting, and weakness within weeks, and in paraesthesia, muscle weakness, cerebral cramps, and cardiac manifestations within months.

The principal sites of dietary magnesium absorption is the small bowel with smaller amounts absorbed in the colon. Intestinal magnesium absorption occurs via (1) a saturable transcellular active pathway and (2) a nonsaturable paracellular passive transport, the latter dependent on the electrochemical gradient (14,15). Saturation of the transcellular absorption at high luminal magnesium concentrations is best explained by the limited transport capacity of active transport (Figure 1A). At low intraluminal magnesium concentrations, magnesium is transported mainly by the active transcellular pathway and with rising concentrations by the paracellular pathway (Figure 1B), yielding a curvilinear function for total absorption.

In the kidney, magnesium transport differs in quantity and kinetics depending on the nephron segment (Figure 2). Fifteen to twenty percent of the ultrafiltrable magnesium is reabsorbed in the proximal tubule of the adult kidney. In the premature kidney of the newborn, proximal tubules can absorb up to 70% (16). In the mature kidney, the majority of magnesium is reabsorbed in the loop of Henle, especially in the cortical thick ascending limb (cTAL). Here, 70% of the ultrafilterable magnesium is normally reabsorbed. With the help of animal models, it was demonstrated that this transport is passive and paracellular, driven by the lumen-positive electrochemical gradient characteristic of this nephron segment (Figure 3A). On the contrary, magnesium reabsorption in the distal convoluted tubule (DCT) is of transcellular and active nature (Figure 3B). The absorption rate in the DCT (5 to 10%) is much lower than in the cTAL, but it defines the final urinary excretion, as there is no significant magnesium reabsorption in the collecting duct. Three to five percent of the filtered magnesium is finally excreted in the urine. Distal tubule magnesium transport has been recently reviewed in detail by Dai et al. (17).

In the following, we will discuss the clinical and genetic aspects of hereditary magnesium-losing disorders. To facilitate this task, we will deal with the following groups of disorders: (1) primary isolated magnesium loss; (2) familial hypomagnesemia with hypercalciumia and nephrocalcinosis (FHHNC); (3) hypomagnesemia with secondary hypocalcemia (HSH); (4)
Ca^{2+}/Mg^{2+}-sensing receptor (CASR)–associated disorders; and (5) disorders associated with renal salt-wasting and hypokalemic metabolic alkalosis, comprising the Gitelman syndrome and the Bartter-like syndromes. An overview on the genetic aspects of these disorders is given in Table 1, an overview on clinical aspects in Table 2.

**Isolated Magnesium Loss**

**Isolated Dominant Hypomagnesemia (IDH).** IDH follows an autosomal dominant mode of inheritance. It was first described by Geven et al. (18) in 1987 in two Dutch families. The index cases of these two families presented with generalized seizures that led to the detection of primary hypomagnesemia. Interestingly, many other affected members of these families remained asymptomatic. Typically, hypomagnesemia in IDH is associated with hypocalciuria that is phenotypically reminiscent of patients presenting with Gitelman syndrome (GS). But GS patients also have hypokalemic alkalosis as a consequence of renal salt wasting, whereas IDH subjects have no other biochemical abnormalities. Newborns of affected mothers may have severe hypomagnesemia at birth, even though maternal hypomagnesemia is less pronounced and devoid of clinical symptoms (19). Thus, immediate control in the neonatal period with appropriate magnesium supplementation is necessary to avoid the potential risks related to hypomagnesemia.

The results of retention studies after oral administration of $^{28}\text{Mg}^{2+}$ and the effects of magnesium infusions on renal reabsorption indicated that the primary molecular defect resides in the kidney (18). As promising candidate genes for IDH were not available, linkage analysis was performed in the two large families, and a gene locus was mapped to chromosome 11q23.
Extended haplotype analysis strongly suggested a common ancestor in the two families. Within the critical interval, Meij et al. identified the gene FXYD2 coding for the \( \gamma \)-subunit of the \( \text{Na}^{+}\text{-K}^{+}\text{-ATPase} \). This subunit is localized to the DCT segment of the nephron, the site of active magnesium transport (21). Sequence analysis showed a heterozygous Gly41Arg mutation in all affected individuals, replacing a highly conserved amino acid residue within the single transmembrane domain of the \( \gamma \)-subunit (22). Meij et al. reported that two individuals with large heterozygous 11q23.3-ter genomic deletions, which comprise the FXYD2 gene locus, did not present with hypomagnesemia (22). This points to a dominant-negative effect of the Gly41Arg mutation rather than to simple haploinsufficiency. It was demonstrated by expression studies in mammalian renal tubule cells (COS-1) that this amino acid exchange results in a misrouting of the \( \gamma \)-subunit.

### Table 1. Inherited disorders or magnesium handling

<table>
<thead>
<tr>
<th>Disorder</th>
<th>OMIM #</th>
<th>Inheritance</th>
<th>Gene Locus</th>
<th>Gene</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated dominant hypomagnesemia with hypocalciuria</td>
<td>154020</td>
<td>AD</td>
<td>11q23</td>
<td>FXYD2</td>
<td>( \gamma )-subunit of the ( \text{Na}^{+}\text{-K}^{+}\text{-ATPase} )</td>
</tr>
<tr>
<td>Isolated recessive hypomagnesemia with normocalciuria</td>
<td>154020</td>
<td>AD</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Familial hypomagnesemia with hypercalciuria/ nephrocalcinosis</td>
<td>603959</td>
<td>AR</td>
<td>3q27-29</td>
<td>CLDN16</td>
<td>paracellin-1, tight junction protein</td>
</tr>
<tr>
<td>Hypomagnesemia with secondary hypocalcemia</td>
<td>602014</td>
<td>AR</td>
<td>9q22</td>
<td>TRPM6</td>
<td>TRPM6, putative ion channel</td>
</tr>
<tr>
<td>Autosomal dominant hypoparathyroidism</td>
<td>600189</td>
<td>AD</td>
<td>3q13.3-21</td>
<td>CASR</td>
<td>CASR, ( \text{Ca}^{2+}/\text{Mg}^{2+} ) sensing receptor</td>
</tr>
<tr>
<td>Antenatal Bartter syndrome/ hyperprostaglandin E syndrome</td>
<td>241200</td>
<td>AR</td>
<td>15q15-21</td>
<td>SLC12A1</td>
<td>NKCC2, ( \text{Na}^{+}\text{-K}^{+}\text{-2Cl}^{-} ) cotransporter</td>
</tr>
<tr>
<td>Classic Bartter syndrome</td>
<td>602023</td>
<td>AR</td>
<td>1p36</td>
<td>CLCNKB</td>
<td>CLC-Kb, distal tubule chloride channel</td>
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<tr>
<td>Antenatal Bartter syndrome with sensorineural deafness</td>
<td>602522</td>
<td>AR</td>
<td>1p31</td>
<td>BSND</td>
<td>Barttin, chloride channel ( \beta ) subunit</td>
</tr>
<tr>
<td>Gitelman syndrome</td>
<td>263800</td>
<td>AR</td>
<td>16q</td>
<td>SLC12A3</td>
<td>NCCT, ( \text{Na}^{+}\text{-Cl}^{-} ) cotransporter</td>
</tr>
</tbody>
</table>

### Table 2. Clinical and biochemical characteristics

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Age at Onset</th>
<th>Serum Mg(^{2+})</th>
<th>Serum Ca(^{2+})</th>
<th>Serum K(^{+})</th>
<th>Blood pH</th>
<th>Urine Mg(^{2+})</th>
<th>Urine Ca(^{2+})</th>
<th>Nephrocalcinosis</th>
<th>Renal Stones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated dominant hypomagnesemia with hypocalciuria</td>
<td>Childhood</td>
<td>↓</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>↑</td>
<td>↓</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Isolated recessive hypomagnesemia with normocalciuria</td>
<td>Childhood</td>
<td>↓</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>↑</td>
<td>N</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Familial hypomagnesemia with hypercalciuria/nephrocalcinosis</td>
<td>Childhood</td>
<td>↓</td>
<td>N</td>
<td>N</td>
<td>or ↓</td>
<td>↑↑</td>
<td>↑↑</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Hypomagnesemia with secondary hypocalcemia</td>
<td>Infancy</td>
<td>↓↓↓</td>
<td>↓</td>
<td>N</td>
<td>N</td>
<td>↑</td>
<td>N</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Autosomal dominant hypoparathyroidism</td>
<td>Infancy</td>
<td>↓↓↓</td>
<td>↓</td>
<td>N</td>
<td>N or ↓</td>
<td>↑↑</td>
<td>↑↑</td>
<td>Yes(^a)</td>
<td>Yes(^a)</td>
</tr>
<tr>
<td>Antenatal Bartter syndrome/ hyperprostaglandin E syndrome</td>
<td>Neonatal</td>
<td>N</td>
<td>N</td>
<td>↓↓↓</td>
<td>↑</td>
<td>N</td>
<td>↑↑</td>
<td>Yes</td>
<td>?</td>
</tr>
<tr>
<td>Classic Bartter syndrome</td>
<td>Infancy</td>
<td>N or ↓</td>
<td>N</td>
<td>↓↓↓</td>
<td>↑</td>
<td>N</td>
<td>to ↑</td>
<td>Variable</td>
<td>Rare</td>
</tr>
<tr>
<td>Antenatal Bartter syndrome with sensorineural deafness</td>
<td>Neonatal</td>
<td>N</td>
<td>N</td>
<td>↓↓↓</td>
<td>↑</td>
<td>N</td>
<td>N to ↑</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Gitelman syndrome</td>
<td>Variable</td>
<td>↓</td>
<td>N</td>
<td>↓↓↓</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

\(^a\) Frequent complication under therapy with calcium and vitamin D.
that the complete ATPase complex might be affected (22). However, subsequent studies in mammalian cells demonstrated that the Gly41Arg mutation only disturbs trafficking of the γ-subunit gamma but not αβ-subunits to the cell surface (23). Thus, the impaired trafficking of the mutant γ-subunit seems to abrogate its functional effects on the αβ-subunits rather than to impair trafficking of the multimeric Na\(^{+}\)-K\(^{+}\)-ATPase. More recently, Meij et al. demonstrated that the Gly 41 Arg mutant FXYD2 is retarded in the Golgi complex, pointing to a disturbed post translational processing (24). Why a mutation in the γ-subunit would result in selective renal magnesium wasting is unknown. The normal γ-subunit decreases sodium affinity and increases ATP affinity to the Na\(^{+}\)-K\(^{+}\)-ATPase enzyme that should increase intracellular sodium concentration and lead to more energy efficient sodium transport (23). Accordingly, diminished basolateral Na\(^{+}\)/Mg\(^{2+}\) exchange with malfunctioning γ-subunit cannot be invoked for a decrease in magnesium transport (17). Meij et al. have suggested that diminished intracellular potassium may depolarize the apical membrane, resulting in a decrease in magnesium uptake (24). Further studies are needed to clarify this issue. It is also unknown why there is an increase in distal calcium reabsorption and hypocalciuria in these patients.

Interestingly, IDH seems to be genetically heterogenous, because FXYD2 has been excluded in a large Californian family with a phenotype very similar to that seen in the Dutch families (25). This may suggest a different genetic disease converging on the same transport function.

**Isolated Recessive Hypomagnesemia (IRH).** Isolated autosomal recessive primary hypomagnesemia was originally described in a consanguinous family (26). The affected individuals presented with symptoms of hypomagnesemia early during infancy. Hypomagnesemia due to increased urinary magnesium excretion appears to be the only abnormal biochemical finding. IRH is distinguished from the autosomal dominant form by the lack of hypocalciuria. Linkage analyses have excluded thus far all established gene loci involved in hereditary hypomagnesemia indicating a different genetic disease (24).

**Familial Hypomagnesemia with Hypercalciuria and Nephrocalcinosis (FHHNC)**

In 1972, Michelis et al. (27) first described a hypomagnesemic disorder characterized by excessive renal magnesium and calcium wasting. In addition, affected individuals had bilateral nephrocalcinosis and developed progressive renal failure. Subsequent clinical descriptions allowed the characterization of the whole clinical spectrum of this disease and enabled this entity to be discerned from other magnesium-losing tubular disorders (28–32). FHHNC patients usually present during early childhood with recurrent urinary tract infections, polyuria/polydipsia, isosthenuria, and renal stones. Some children come to the attention of physicians with failure to thrive, vomiting, abdominal pain, tetanic episodes, or generalized seizures. Besides hypomagnesemia, biochemical abnormalities include hypermagnesiuria and hypercalciuria, and impaired GFR is often detected at the time of diagnosis. Patients also tend to have increased parathyroid hormone (PTH) levels preceding the impairment of GFR. A substantial percentage of patients show incomplete distal renal tubular acidosis, hypochloremia, and hyperuricemia. Extrarenal manifestations, such as ocular involvement (including severe myopia, nystagmus, and choriorretinitis), have been repeatedly reported (30,31).

In addition to continuous magnesium supplementation, therapy aims at the reduction of calcium excretion by using thiazides to prevent the progression of nephrocalcinosis and stone formation, because the degree of renal calcification has been correlated with progression of chronic renal failure (CRF) (31). However, it seems that current therapeutic approaches do not significantly influence the progression of renal failure. In a large series of 33 patients, one third had already developed end-stage renal disease early during adolescence (33). Hypomagnesemia may completely disappear with the decline of GFR due to the reduction of the filtered magnesium load. Renal transplantation corrects the abnormal magnesium and calcium handling clearly demonstrating that the primary defect resides in the kidney.

Based on clinical observation and clearance studies, Rodriguez-Soriano et al. postulated that the primary defect in FHHNC was related to impaired magnesium and calcium reabsorption in the loop of Henle. Using a positional cloning approach, Simon et al. identified ten different mutations in a novel gene (CLDN16, formerly PCLN-1) on chromosome 3q27–29 in the affected individuals of ten FHHNC families (34). CLDN16 mutations as the underlying cause of FHHNC have subsequently been confirmed in additional patients (33,35). In addition, a common mutation due to a founder effect was characterized in FHHNC patients originating from Germany and Eastern European countries (33). As this mutation is present in approximately 50% of the mutant alleles, molecular diagnosis is greatly facilitated in patients originating from these countries.

CLDN16 codes for paracellin-1 (claudin-16), a member of the claudin family of tight junction proteins. The claudin family comprises a set of structurally related proteins involved in the formation of tight junction strands in various tissues (36). RT-PCR studies on microdissected nephron segments and immunohistochemical studies demonstrated that CLDN16 is strongly expressed both in the medullary and cortical segments of the loop of Henle in human and rodent kidneys. It was shown by immunohistochemistry that paracellin-1 colocalized with occludin at tight junctions (34). These data indicate that paracellin-1 is an important component of tight junction formation in the thick ascending limb. Impaired expression of paracellin-1 is associated with severe renal calcium and magnesium loss without loss of other electrolytes; it is therefore speculated that paracellin-1 contributes to the formation of calcium and magnesium selective paracellular pathways. This hypothesis is supported by the recent observation that two other claudins (CLDN4 and CLND15) influence ion selectivity by creating charge-selective channels through the tight-junction barrier (37,38). Hydrophobicity analysis predicts that paracellin-1 is a four-transmembrane domain protein with intracellular N- and C-termini. The C-terminus has a C-terminal PDZ
domain, which is possibly involved in protein-protein interactions. The first extracellular loop is highly negatively charged, which distinguishes paracellin-1 from other members of the claudin family. It is proposed that the pathway formed by paracellin-1 involves this first extracellular loop and that its negative charge contributes to the observed cation sensitivity. The longest open reading frame of the human cDNA encodes a protein of 305 amino acids with a cytoplasmatic N-terminus of 73 amino acids. This structure is in contrast to all other claudins that share a very short N-terminus of only six or seven amino acids. Interestingly, there is a second inframe start codon within a suitable Kozak consensus sequence at position Met71, which is analogous to all other claudins. Sequence comparison of the human cDNA with other species and the results of mutation analysis suggest that the second translation initiation start site is used in vivo (33,39,40).

A large deletion of CLDN16 has also been shown to underlie the development of a chronic interstitial nephritis in Japanese cattle that rapidly develop CRF shortly after birth (39,41). Interestingly, the typical electrolyte abnormalities seen in FH-HNC were not detected in affected animals. They typically show hypocalcemia but no hypomagnesemia. This is probably due to the advanced CRF present at the time of examination. In this context, it is important to note that the majority of mutations reported so far in FHHNC are simple missense mutations affecting the transmembrane domains and the extracellular loops. Thus, the disease phenotype resulting from a large deletion in CLDN16 might be expected to be more pronounced with early onset renal failure, whereas point mutations might primarily affect calcium and magnesium reabsorption with progressive renal failure as a secondary event. However, Cldn16 knockout mice did not show renal failure during the first months of life (42). Referring to the ocular abnormalities observed in some FHHNC patients, it is interesting to note, that Meij et al. found CLDN16 expression in bovine cornea and retinal pigment epithelia (43). Examination of the eyes of affected Japanese cattle and of the Cldn16 knockout mice might give an answer to the question whether myopia, nystagmus, and choioretinitis observed in humans are directly linked to CLDN16 mutations.

There is evidence from family analyses that carriers of heterozygous CLDN16 mutations may also present with clinical symptoms. Two independent studies describe a high incidence of hypercalciuria, nephrolithiasis, and nephrocalcinosis in first-degree relatives of FHHNC patients (31,33). In a subsequent study, Blanchard et al. (35) also found a tendency toward hypercalciuria or mild hypomagnesemia in family members with heterozygous CLDN16 mutations. Thus, one might speculate that CLDN16 mutations are involved in idio-pathic hypercalciuric stone formation. Very recently, a homozygous CLDN16 mutation (Thr303Gly) affecting the C-terminal PDZ domain has been identified in two families with isolated hypercalciuria and nephrocalcinosis without disturbances in renal magnesium handling (44). Interestingly, hypercalciumia disappeared during follow-up and reached normal values beyond puberty. Transient transfection of MDCK cells with the Thr303Gly mutant CLDN16 revealed the localization of mutant paracellin-1 in the apical membrane, whereas wild-type paracellin-1 was correctly targeted to the basolateral membrane (44). It still remains to be determined why this type of misrouting is associated with transient isolated hypercalciuria without increased magnesium excretion.

The identification of the molecular components involved in transport across renal epithelial cells via the paracellular pathway has already considerably increased our current knowledge of these processes. It might be expected that the analysis of other components, such as occludins or other claudins expressed in tubular epithelia, will further contribute to the understanding of the physiologic functions but also of disease states related to impaired paracellular reabsorption.

**Hypomagnesemia with Secondary Hypocalciuria (HSH)**

HSH or primary intestinal hypomagnesemia is an autosomal recessive disorder that is characterized by very low serum magnesium and low calcium levels. It was first described by Paunier et al. in 1968 (45). Patients usually present within the first 3 mo of life with neurologic symptoms of hypomagnesemic hypocalcemia, including seizures, tetany, and muscle spasms. Untreated, the disorder may result in permanent neurologic damage or may be fatal. Hypocalcemia is secondary to parathyroid failure and peripheral parathyroid hormone resistance as a result of sustained magnesium deficiency (46). Usually, the hypocalcemia is resistant to calcium or vitamin D therapy. Normocalcemia and relief of clinical symptoms can be attained by administration of high oral doses of magnesium, up to 20 times the normal intake (47). As large oral amounts of magnesium may induce severe diarrhea and noncompliance in some patients, parenteral magnesium administration has sometimes to be considered. Alternatively, continuous nocturnal nasogastric magnesium infusions have been proven to efficiently reduce gastrointestinal side effects (48). The pathophysiology of HSH was largely unknown, but physiologic studies pointed to a primary defect in saturable intestinal magnesium transport (49). In some patients, an additional renal leak of magnesium, probably caused by altered magnesium entry into epithelial cells of the DCT was suspected (13,50).

Using a DNA pooling strategy, a gene locus (HOMG1) for HSH was mapped to chromosome 9q22 in a large inbred Bedouin kindred (51). It was further refined to a critical interval of less than 1 centimorgan (52). Recently, Schlingmann et al. (53) and Walder et al. (54) identified mutations in a novel gene, TRPM6, in several unrelated HSH families as the underlying cause of HSH. TRPM6 encodes a new putative ion channel belonging to the transient receptor potential (TRP) channel family. TRP ion channels are characterized by six transmembrane segments, a highly conserved putative pore-forming region, and a Pro-Pro-Pro motif following the last transmembrane segment (55). Within the TRP family, TRPM6 belongs to the TRPM subclass, whose members share a long conserved specific N-terminal domain of unknown function. It shows highest homology with TRPM7, which has recently been identified as a magnesium and calcium permeable ion channel regulated by Mg$^{2+}$ATP (56). In addition to the ion channel domain, TRPM6 and TRPM7 have a C-terminal re-
gion with sequence similarity to serine-threonine protein kinases of the atypical α-kinase family, therefore representing a new class of bifunctional proteins (57). Whether this kinase domain is necessary for the channel function is still unclear. By RT-PCR and in situ hybridization, TRPM6 expression has been demonstrated along the entire small and large intestinal tract and in distinct distal tubule segments representing DCT cells (53).

The observation that HSH patients may achieve normal serum magnesium levels by high oral magnesium intake supports the theory of two independent pathways for intestinal magnesium absorption. TRPM6 probably represents a molecular component of the active transcellular pathway so that high oral doses of magnesium are sufficient to compensate the defect of TRPM6-mediated transport by increasing magnesium absorption through the paracellular pathway.

The detection of TRPM6 expression in the DCT substantiated the hypothesis advanced by Cole and Quamme of an additional role of impaired renal magnesium reabsorption in HSH pathophysiology (13). This was confirmed by intravenous magnesium loading studies in HSH patients, who clearly showed a considerable renal magnesium leak, even under hypomagnesemic conditions (54).

\( \text{Ca}^{2+}/\text{Mg}^{2+} \)-Sensing Receptor–Associated Disorders

An important regulator of the magnesium homeostasis is the \( \text{Ca}^{2+}/\text{Mg}^{2+} \)-sensing receptor (CASR), which was identified in 1993 by Brown, Hebert, and colleagues (58). The CASR is a member of the G protein-coupled receptor family, which forms dimers through interactions of the cysteine residues of the extracellular domain (59). CASR is located in the apical membrane of PTH-secreting cells of the parathyroid glands and basolaterally in the nephron segments, i.e., TAL and DCT involved in renal calcium and magnesium reabsorption (60,61). It also has been detected in tissues not primarily involved in calcium homeostasis, among others in the apical membrane of the inner medullary collecting duct, where it probably contributes to the inhibitory effect of hypercalcemia on vasopressin-stimulated water reabsorption.

An important function of the CASR is to sense ionized serum calcium and magnesium concentrations and to regulate these levels by controlling PTH secretion. In the past decade, both activating and inactivating mutations of the CASR have been described, resulting in different clinical entities. Familial hypocalciuric hypercalcemia (FHH) and neonatal severe hyperparathyroidism result from inactivation mutations present in a either heterozygous or homozygous (or compound heterozygous) state (62,63). In addition to hypercalcemia, FHH patients also have a tendency to elevated serum magnesium levels (64).

Activating mutations of the CASR gene were first described in families affected with autosomal dominant hypocalcemia (ADH) (65,66). Affected individuals present with hypocalcemia, hypercalciuria, and polyuria, and about 50% of these patients have hypomagnesemia (66,67). Hypocalcemia in ADH is generally mild to moderate; severe hypocalcemia has rarely been reported in this syndrome. Carpopedal spasms and/or seizures are typical symptoms but ADH is also sometimes asymptomatic.

Activating mutations shift the setpoint of the receptor to a level of enhanced sensitivity by increasing the apparent affinity of the mutant receptor for extracellular \( \text{Ca}^{2+} \) and \( \text{Mg}^{2+} \). This results in diminished PTH secretion and decreased reabsorption of divalent cations in the cTAL and DCT, which leads to loss of urinary calcium and magnesium (68). The inhibition of calcium and magnesium reabsorption in the loop of Henle is thought to be due to a selective reduction of the paracellular permeability and/or to the reduction of the luminal-positive transepithelial voltage by inhibiting the transcellular NaCl reabsorption (68). Hormone-stimulated magnesium reabsorption is also inhibited in the DCT that probably contributes to a greater extent to renal magnesium loss than does its action in the loop (17).

After vitamin D or calcium therapy, ADH patients dramatically increase urinary calcium excretion even though serum calcium levels may be still in the low-normal range (66). This may lead to polyuria, nephrocalcinosis, nephrolithiasis, and even irreversible reduction of renal function. Therefore, the treatment of hypocalcemia in ADH with vitamin D and calcium supplementation should be restricted to symptomatic patients.

Very recently, three additional patients with ADH due to activating CASR mutations have been described (69,70). During follow-up, the clinical course in these patients was complicated by a Bartter-like syndrome, i.e., the patients developed renal salt and water loss associated with hypokalemic metabolic alkalosis. In addition, all three patients had hypomagnesemia (69,70). Heterologous expression of the mutant CASR revealed that the underlying mutations (L125P, C131W, A843E) figure among the most potent gain-of-function CASR mutations reported so far. These mutations appear to be fully activated under normal serum calcium concentrations and are able to induce a significant loss of NaCl by inhibiting the reabsorption of NaCl in the TAL and thus patients present with a Bartter-like syndrome.

Salt-Losing Tubular Disorders

Several disorders have been described that have in common the cardinal symptoms of renal salt wasting, hypokalemic metabolic alkalosis, and elevated plasma renin and aldosterone levels but normal BP. On the basis of clinical presentation, additional symptoms, and the biochemical profile, especially with respect to calcium and magnesium handling, at least four different disease entities can be discerned (71,72). Over the last few years, this clinical differentiation has been substantiated by the characterization of the underlying molecular defects in five different genes in patients suffering from these diseases.

Antenatal Bartter Syndrome or Hyperprostaglandin E Syndrome (aBS/HPS). Antenatal Bartter Syndrome or hyperprostaglandin E syndrome (aBS/HPS) is characterized by massive polyuria that manifests in utero with the development of polyhydramnios that results in premature birth in almost all cases. Postnatally affected infants rapidly develop salt wasting and hypokalemic metabolic alkalosis. In addition, hypercalci-
ura and pronounced medullary nephrocalcinosis occur in all affected individuals (1). Magnesium wasting is not a common finding in aBS/HPS (73). As these patients fail to respond to furosemide, a defective NaCl reabsorption in the thick ascending limb was suspected (74). By combining a candidate gene approach with linkage analysis, Simon et al. demonstrated that aBS/HPS is either due to mutations in the Na⁺⁻K⁺⁻Cl⁻ co-transporter (NKCC2) or to the apical potassium channel ROMK, which is necessary for proper NKCC2 function in the TAL (75,76).

Mutations in NKCC2 or ROMK are thought to reduce salt reabsorption in the TAL and thus should impair not only transepithelial calcium but also magnesium reabsorption. However, renal magnesium wasting and overt hypomagnesemia do not reliably segregate with the aBS/HPS phenotype. This is consistent with the observation of normal magnesium homeostasis in NKCC2 knockout mice (77). Also chronic furosemide treatment is not generally associated with hypomagnesemia. Possibly, the loop of Henle or more distal parts of the nephron may adapt and compensate more efficiently for magnesium relative to calcium explaining the presence of hypercalciuria without overt magnesium loss in this syndrome. Increased renal prostaglandin synthesis, which is generally observed in aBS/HPS, may contribute to increased magnesium reabsorption in the DCT, as has been demonstrated for PGE₂ in a mouse DCT cell line (78). Alternatively, chronic volume depletion could stimulate passive magnesium reabsorption in the proximal tubule and TAL. In TAL, this adaptation might involve paracellin-1 so that more magnesium is absorbed at a lowered transepithelial potential difference.

Classic Bartter Syndrome (cBS). Classic Bartter Syndrome (cBS) usually presents during infancy or early childhood, and the phenotype of these patients best fits with the original description given by Bartter et al. (79), i.e., without prenatal onset and without nephrocalcinosis seen in the antenatal variant. cBS is caused by mutations in CLCNKB encoding the basolaterally located renal chloride channel ClC-Kb, which mediates chloride efflux from the tubular epithelial cell to the interstitium along the TAL and DCT (80,81). The broad expression pattern of ClC-Kb might explain the wide clinical spectrum of cBS phenotypes ranging from that similar to aBS/HPS in rare cases to those almost identical to Gitelman syndrome (73) (see below). An alternative explanation could be an inter-individual difference in the ability to compensate for a defect in basolateral chloride transport, e.g., by activation of alternative chloride efflux pathways such as the K⁺⁻Cl⁻ cotransporter (82). Hypomagnesemia is detected in up to 50% of patients with mutations in CLCNKB (81) and calcium excretion is variable, but hypocalciuria is not an unusual finding (73).

Antenatal Bartter Syndrome with Sensorineural Deafness (BSND). Landau et al. (72) first described a subtype of antenatal BS that is characterized by a similar clinical phenotype but which is associated in all cases with sensorineural deafness. The renal phenotypic presentation is even more severe than in aBS/HPS individuals with massive salt and fluid loss from birth, that often needs long-term parenteral fluid supplementation. Hypercalciuria and nephrocalcinosis are uncommon, and the response to indomethacin therapy is poor. In addition, many patients develop progressive renal failure of unknown etiology (83). By homozygosity mapping in a large Bedouin kindred, Brennan et al. mapped this disease to chromosome 1p31 (84). Within the critical interval, a new gene (BSND) coding for a protein named Barttin was identified (85). Barttin has no similarity with any known protein and does not share any similarities with any ion channel. Consistent with the characteristic phenotypic presentation, Barttin is expressed along the kidney tube and in the stria vascularis (marginal cells) of the inner ear. Functional expression studies revealed, that Barttin is a beta-subunit of the renal chloride channels (ClC-Ka and -Kb in humans, ClC-K1 and -K2 in mice) (86,87).

Interestingly, in contrast to cBS due to CLCNKB mutations, hypomagnesemia has not been reported in BSND individuals. The reason for this is unknown, but the decreased GFR in BSND patients might, at least in part, prevent the development of magnesium wasting. Alternatively, as in aBS/HPS, prostaglandin formation is also highly stimulated in BSND patients so that increases in magnesium reabsorption in the DCT might mitigate magnesium losses.

Gitelman Syndrome (GS). GS was first described by Gitelman et al. in 1966 (88). Salt and water losses in patients with GS are less pronounced than in aBS/HPS or cBS because their urinary-concentrating ability is preserved to a greater extent. They usually present during childhood or adolescence, even if the biochemical abnormalities may be detected earlier. Cardinal symptoms include muscle weakness or tetanic episodes that are related to profound hypomagnesemia. In addition, GS patients virtually always have hypocalciuria. The presence of both hypomagnesemia and hypocalciuria is highly predictive for the diagnosis of GS (89) even if in rare cases this combination is also detected in CBS patients (90). GS is often described as the mild variant of the salt-losing tubular disorders, and many asymptomatic patients have been reported. However, this notion is probably not true. Recently, Cruz et al. demonstrated that GS affects quality-of-life to the same degree as hypertension or diabetes for example (91). None of the 50 GS patients of this study were truly asymptomatic. Salt craving, nocturia, and paraesthesia were among the most frequent symptoms. The authors did not find any correlation between the symptoms reported and either potassium or magnesium levels.

The dissociation of renal magnesium and calcium handling together with the observed unresponsiveness to thiazides pointed to a primary defect in the DCT. Again, using a positional-candidate gene approach, Simon et al. identified putative loss of function mutations in the gene coding for the NaCl cotransporter (NCCT) of the DCT (92). GS appears to be a genetically homogenous disorder, and more than 100 different mutations have been reported to date. Results from heterologous expression of naturally occurring GS mutations in Xenopus oocytes revealed that the majority of the mutations are retained in the endoplasmic reticulum, resulting in impaired trafficking to the plasma membrane (93). Other mutant pro-
teins were shown to be inserted in the plasma membrane, but sodium transport was considerably reduced (94).

Schultheis et al. created a transgenic mouse model that completely lacked the NCCT by disrupting the SLC12A3 gene (95). These mice display all the cardinal features of GS, especially hypomagnesemia and hypocalciuria, but hypokalemia was not observed. Hypocalciuria in GS is explained by the reduced entry of NaCl into the DCT cell, leading to hyperpolarization. Cellular hyperpolarization increases calcium reabsorption mediated by an apical entry via an epithelial calcium channel and basolateral extrusion through the Na\(^+\)/Ca\(^2+\) exchanger that could explain the hypocalciuria. The reason for the often-pronounced hypomagnesemia in GS is still unknown. Chronic thiazide therapy, a model for GS, does not uniformly lead to magnesium wasting and hypomagnesemia. The NCCT knockout model does not favor a common hypothesis that hypomagnesemia is secondary to the hypokalemia observed in GS. Reilly and Ellison speculated that magnesium may back-flux through a paracellular pathway in the DCT (96,97). This hypothesis is based on the assumption that in GS the DCT cells are converted from mainly electroneutral cells to cells that reabsorb NaCl in an electrogenic manner via the epithelial sodium channel ENaC, and the action of aldosterone, which normally stimulates NaCl reabsorption by NCCT, would increase the magnitude of the lumen-negative voltage. This, in turn would favor paracellular secretion of magnesium and potassium. Reilly and Ellison proposed that paracellin-1 could be involved in this paracellular pathway, because in the initial report, RT-PCR showed paracellin-1 expression in the DCT (34). Recent data analyzing the expression profile of several claudins along the nephron by immunofluorescence microscopy could not detect paracellin-1 in the DCT (98). In addition, earlier microperfusion studies failed to detect any magnesium secretion in the distal tubule (99). An alternative explanation comes from studies in rats receiving chronic thiazide treatment. Loffing et al. showed that a complete block of NCCT results in an increased rate of apoptosis in DCT cells (100). This is consistent with the observation of Schultheis et al. in the NCCT knockout model, who also demonstrated a decrease in number, height, and basolateral infolding of DCT cells (95). Perhaps diminished absorptive surface area of the DCT in GS compromises magnesium reabsorption to such an extent that magnesium wasting ensues. The reason for hypomagnesemia in GS is an ongoing debate that needs further research activities.

In summary, inherited disorders of magnesium metabolism represent a heterogenous group of diseases with complex phenotypes. This is partly due to the simultaneous disturbance of other electrolyte systems, especially of calcium and potassium homeostasis.

The proteins underlying the pathogenesis of these disorders demonstrate a surprising variety:

- The \(\gamma\)-subunit of the enzyme Na\(^+\)-K\(^+\)-ATPase
- Paracellin-1, a tight-junction protein mediating paracellular transport
- TRPM6, a novel channel with magnesium-conducting properties
- The CASR, a member of the G protein-coupled receptor family
- NCCT, a sodium chloride cotransporter
- The chloride channel CIC-Kb

The characterization of corresponding transgenic animal models has proven to be extremely helpful to study the physiology of the newly identified proteins. Further investigations on these animal models will hopefully give more insight into regulatory mechanisms, new therapeutic approaches, and the consequences of long-term follow-up. Advances in molecular modeling will possibly promote the development of new drugs acting specifically on the novel components of magnesium metabolism, for example inhibitors of the CASR, activating agents for TRPM6, NKCC2, and NCCT, or even chaperone-like drugs, directing the trafficking of misrouted proteins.

The association of hypomagnesemia with diabetes mellitus type II, arterial hypertension, coronary heart disease, and asthma bronchiale challenges further research on genetic variants in genes underlying primary hypomagnesemia.

Genetic variations of these genes might influence disease manifestation, progression, and clinical outcome. It could be imagined, that carriers of polymorphisms or constellations of polymorphisms have a predisposition to develop one of the mentioned diseases. As these chronic diseases affect a high percentage of the population, this is of great socioeconomic impact.

The results of mutation analysis in familial hypomagnesemia demonstrate that there is still a number of patients with an unidentified genetic defect both of recessive and dominant pattern of inheritance (24,25). Thus, it seems likely that new genes involved in magnesium transport mechanisms will be identified in the near future adding to our current knowledge on magnesium metabolism.

Acknowledgments

We thank Gary Quamme, Nikola Jech, Melanie Peters, and Siegfried Waldegger for their critical reading of the manuscript and the helpful discussions during its preparation. This work was supported by the Deutsche Forschungsgemeinschaft (Ko1489–3/2).

References


