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Genetic disorders of magnesium homeostasis

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Introduction

Magnesium plays an essential role in a wide variety of biological activities (Wacker 1980). One very important function is that of cofactor in muscle contraction, and it is not surprising therefore that many symptoms of magnesium deficiency are related to muscle dysfunction (Table 1). The major direct risk of hypomagnesemia is myocardial ischemia (Kelepouris & Agus 1998). Genetic defects leading to hypomagnesemia have been shown to result in either intestinal malabsorption or renal reabsorption problems. In this review we will give a clinical and genetic overview of the current insights in different forms of familial hypomagnesemia and discuss the putative correlations of the genetic defects with associated electrolyte disturbances.

Normal serum magnesium concentration (0.75–1.4 mmol $l)^{-1}$ is balanced by changes in urinary magnesium excretion in response to altered uptake by the intestine. The main site of absorption is the small bowel. Some additional absorption takes place in the large bowel (Agus 1999). Regulation and fine-tuning of serum magnesium concentration occurs primarily in the kidney, as described in the article by Romero in this issue.

Genetic disorders of magnesium tranport

At present, 4 different disease entities with primary hypomagnesemia have been described (Table 2) three of which are also associated with a disturbance in Ca^{2+}-homeostasis (Meij et al. 2000a). Primary hypomagnesemia can be caused either by an intestinal or by a renal defect. Both intestinal and renal defects can be partially corrected by oral Mg^{2+}-supplements. However, excessive supplementation may lead to diarrhea. For the renal defect, patients both responsive and non-responsive (Geven *et al.* 1987a, 1987b; Praga 1995) to oral Mg^{2+}-supplementation have been described. As is the case in the kidney, absorption in the intestine can be via either the active (transcellular) or the passive (paracellular) absorption pathway. If either route is impaired, uptake of Mg^{2+} through the unimpaired route is enhanced to compensate for the defect in the other pathway. To differentiate between the intestinal and the renal defect 24 h urinary Mg^{2+}-excretion or the fractional excretion in a random urine sample should be determined. Mg^{2+}-excretion exceeding 10–30 mg 24 h or a fractional excretion above 2% in a random sample points to a renal defect. Alternatively, tracing of orally administered $^{28}Mg^{2+}$ or an intravenous Mg^{2+}-load can determine whether the intestinal absorption or the tubular reabsorption is disturbed.

The only intestinal form of hypomagnesemia known thus far is associated with hypocalcemia. Among the renal diseases, a further distinction can be made on the basis of associated disturbances in urinary Ca^{2+}-excretion and through different inheritance patterns, reflecting both the clinical and genetic heterogeneity of these disorders. We will discuss the clinical characteristics of each disease listed in Table 2 and include the most recent genetic insights. Subsequently, some genetic disorders will be discussed in which hypomagnesemia is assumed to occur as a secondary feature.

Primary hypomagnesemia disorders

Intestinal hypomagnesemia with hypocalcemia (HSH)

Hypomagnesemia with secondary hypocalcemia or hypomagnesemic tetany (HSH, OMIM 602014)[1] is caused by an intestinal defect in Mg^{2+}-absorption and was first described in 1965 (Paunier *et al.*) It is characterized by extremely low serum Mg^{2+}-levels (0.15–0.30 mmol l) associated with symptomatic hypocalcemia. Patients typically present between the third

Table 1. Frequent and less frequent symptoms of magnesium deficiency.

Most frequently occurring symptoms	Less frequent manifestations
Epileptiform convulsions	Anorexia, nausea and vomiting
Tetany	Hallucinations
Muscular weakness	Depression
Myopathic potentials on the electromyogram	Agitation
Low voltage T-wave and PQRS-complex, short PR-interval on the EKG	Positive Chvostek and Trousseau signs
Tremulousness	Ataxia
Hyperreflexia	Vertigo
Gross muscular tremor	Apathy
	Delirium
	Chorioform movements

Table 2. Primary hereditary hypomagnesemia disorders.

Disease	Locus	Gene	Reference
Hypomagnesemia with secondary hypocalcemia (HSH)	9q12-9q22.2	?	Walder *et al.* 1997
Familial hypomagnesemia, hypercalciuria and nephrocalcinosis (HHN)	3q28-3q29	*CLDN16*	Simon *et al.* 1999
Dominant hypomagnesemia/hypocalciuria	11q23	*FXYD2*	Meij *et al.* 1999, 2000
Recessive hypomagnesemia/normocalciuria	?[a]	?	Geven *et al.* 1987b

[a] 3q, 9q, and 11q have been excluded (unpublished data).

week and fourth month of life with restlessness, tremor, tetany and overt seizures. Two cases evaluated shortly after birth because of a family history of HSH were found to be hypomagnesemic prior to the onset of clinical symptoms (Skyberg *et al.* 1968; Nordio *et al.* 1971; Lombeck *et al.* 1975) pointing to the primary nature of the hypomagnesemia. Usually, the hypocalcemia does not respond to Ca^{2+}, vitamin D or parathyroid hormone (PTH). During hypomagnesemia, excretion of Mg^{2+} both in urine and stool are normal. High amounts of oral Mg^{2+} can correct the hypocalcemia, probably due to normal functioning of the passive absorption route in the intestine. When supplementation is interrupted, the symptoms of HSH return within 1 to 4 weeks, depending on body Mg^{2+}-stores. Without treatment, the disease is usually fatal within the first year of life.

Generally, more boys than girls are affected by HSH. Therefore, it was initially believed that the disorder is X-linked. The finding of a female patient with a balanced translocation between the X-chromosome and chromosome 9 (t(9;x)(q12;p22)) (Meyer *et al.* 1978) sustained this notion. Walder *et al.* (1997) however, using this patient and 3 additional inbred Bedouin kindreds from Israel, excluded the X-chromosome and found linkage to a 14 cM region on chromosome 9q12–q22. In a follow-up study, two more families with HSH were investigated, which enabled refinement of the linkage interval to a 1 cM region between markers D9S1115 and D9S175 (Walder *et al.* 1999). Previously, the translocation breakpoint on chromosome 9 had been shown to lie within 7.5 cM of marker D9S1115 (Walder *et al.* 1997). As yet, the genetic defect causing HSH remains to be elucidated.

Familial hypomagnesemia with hypercalciuria and nephrocalcinosis

Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (HHN, OMIM 248250) is a progressive renal disease, characterized by hypomagnesemia, hypercalciuria and nephrocalcinosis. Recurrent urinary tract infections and kidney stones are often observed, serum PTH is elevated, and in one third to half of the patients, ocular abnormalities are observed such as horizontal nystagmus, myopia, corneal calcifications and chorioretinitis. Neither chronic oral Mg^{2+} nor thiazide diuretics can correct serum Mg^{2+}- or urinary Ca^{2+}-levels, respectively (Praga *et al.* 1995; Weber *et al.* 2000). It is noteworthy that these patients do not show hypocalcemia, possibly due to increased intestinal absorption and bone release of

Ca^{2+}. Also, additional/other Ca^{2+}-reabsorption pathways in the kidney may partly correct for the urinary loss of Ca^{2+}. The severity of renal calcification correlates with the development of renal failure (Praga *et al.* 1995). Treatment therefore focuses on the prevention of stone formation in these patients (Monnens *et al.* 2000).

HHN is inherited in an autosomal recessive fashion. Remarkably though, both Praga *et al.* (1995) and Weber *et al.* (2000) found a high incidence of hypercalciuria in family members of affected patients. Some also had recurrent nephrolithiasis and in two, mild hypomagnesemia was observed. Finally, in both studies, more females were affected than men (6/8 and 8/13, respectively). In this regard, Praga and colleagues refer to an interesting publication by Koh and Min (1991) describing that in Mg^{2+}-deficient rats which are fed high fructose diets, renal calcifications are considerably more significant in female rats.

Recently, Simon and colleagues (1999) identified the gene involved in HHN. They performed a genome wide linkage study in 3 consanguinous kindreds and found a gene locus on chromosome 3q28–29. Characterization of 9 additional families reduced the linkage interval to a 1 cM region between markers D3S1314 and 539–3. Using a positional cloning strategy the *PCLN-1* gene (OMIM 603959) was cloned. The gene was recently renamed *CLDN16* (GenBank Accession numbers cDNA: AF152101, genomic sequence present in AC009520), and encodes a 305 amino acid protein with sequence and structural homology to the claudin family of tight junction proteins. Claudins are expressed in a tissue specific manner, depending on the species. At present, 24 claudins have been identified. They are able to form selective paracellular pathways by both homo- and heteromeric interactions (Furuse *et al.* 1998; Morita *et al.* 1999; Tsukita *et al.* 1999). The CLDN16 protein is predicted to be a 4 transmembrane domain protein with intracellular N- and C-termini with a highly negatively charged first extracellular loop and a PDZ consensus sequence at the C-terminus. Screening of the gene in the 12 kindreds with HHN revealed 10 different mutations. A year later, Weber *et al.* (2000) identified 6 additional mutations in 8 other HHN families.

The CLDN16 protein is specifically expressed in the kidney in the thick ascending limb (TAL) and distal convoluted tubule (DCT). It was shown to colocalize with occludin in intercellular junctions of human kidney sections (Simon *et al.* 1999). Occludin and claudins are components of tight junction strands, and CLDN16 is believed to be an essential component of the selective paracellular pathway for Mg^{2+}- and to a lesser extent Ca^{2+}-reabsorption in the TAL. Its function in the DCT, where the transepithelial voltage difference does not favour Mg^{2+} reabsorption, is as yet unclear.

In view of the ocular abnormalities in patients with HHN, we investigated the expression of the gene in different structures of the eye. Studies on the corneal epithelium have demonstrated a paracellular permeability barrier (Huang *et al.* 1989), and the CLDN16 protein could have a function in the cornea that is similar to the one in the kidney. Using RT-PCR on different tissues dissected from bovine eye, we indeed found expression of the *cldn16* gene in cornea epithelium and retinal pigment epithelium (unpublished data). It is as yet unclear in which way the ocular findings in HHN patients are explained by *CLDN16* mutations and why ocular abnormalities are not found in all patients.

A functional role for the CLDN16 protein in bovine became apparent when a null mutation (absence of the first four exons) was found in cattle (Hirano *et al.* 2000). In contrast to humans, these animals suffer from chronic interstitial nephritis (CINFH), characterized by defective filtration and reabsorption. Cattle affected with CINFH have hypocalcemia, an effect not seen in human individuals with *CLDN16* mutations.

Renal hypomagnesemia with hypocalciuria

Mg^{2+} wasting associated with hypocalciuria (OMIM 154020) is a renal disorder with an autosomal dominant mode of inheritance. It has some similarities to Gitelman's syndrome (GS). In GS however, hypokalemia and metabolic alkalosis are seen, symptoms which are not present in the two kindreds with dominant renal hypomagnesemia reported by Geven *et al.* (1987a). The two probands of these families were admitted to hospital because of generalized convulsions. Serum Mg^{2+} in both patients appeared to be low (0.39 and 0.40 mmol l, respectively). Values for other electrolytes in the serum, including Ca^{2+}, Na^{+}, K^{+}, Cl^{-} and bicarbonate were all normal, as were blood pH, renin activity and plasma aldosterone. The only additional abnormality related to low serum Mg^{2+} was a lowered renal Ca^{2+} excretion. Surprisingly, affected family members of these probands showed no symptoms of Mg^{2+} deficiency. Tracing of orally administered $^{28}Mg^{2+}$ and the effects of Mg^{2+} infu-

sion on tubular reabsorption pointed to a defect in the kidney.

Recently, we identified the genetic defect in the patients of these two families (Meij *et al.* 2000b). We first performed a genome wide scan and found linkage of the disease with marker *D11S4127* located on chromosome 11q23. Using additional polymorphic markers, we were able to narrow the linkage interval to 5.6 cM. Additionally, we found that the patients of both families shared haplotypes over a region of 10.5 cM, indicating that they are descendants of a common ancestor (Meij *et al.* 1999).

We screened the linkage region for candidate genes with a kidney specific expression pattern, preferably expression in the DCT, the site of active Mg^{2+}-reabsorption. The Na^+, K^+-ATPase γ-subunit is expressed mainly in kidney with the highest expression levels in the DCT and TAL (Mercer *et al.* 1993; Therien *et al.* 1997; Arystarkhova *et al.* 1999). The γ-subunit is encoded by the *FXYD2* (OMIM 601814) gene and has homologies to a family of ion channel inducing proteins (Sweadner & Rael 2000a). The Na^+, K^+-ATPase provides the driving force for active transport processes in the kidney and is responsible for the maintenance of the transmembrane potential and the Na^+-gradient which in turn drive passive reabsorption and facilitated Na^+-coupled transport respectively. Fine mapping of the *FXYD2* gene showed that the gene is located between markers *D11S939* and *D11S4127* which are both within the linkage region (Meij *et al.* 2000b). This warranted a further examination of the *FXYD2* gene in the families with dominant renal hypomagnesemia.

FXYD2 consists of 7 exons coding for two known splice-variants (Kuster *et al.* 2000; Sweadner & Rael 2000a; Meij *et al.* 2000b). Several putative promoter elements have been identified for both transcripts (Sweadner *et al.* 2000b). Mutation detection analysis yielded a G41R mutation (121G $\rightarrow$ A) which resulted in the replacement of a highly conserved amino-acid with a charged arginine residue within the single transmembrane domain of the protein. The mutation cosegregated with the disease in both families and was not found in 132 control chromosomes. We performed expression studies in both insect Sf9 cells and mammalian COS-1 cells and showed that the normal routing to the plasma membrane of the γ-subunit is clearly disturbed in both cell types (Figure 1). Moreover, when we coexpressed the α- and β-subunit of the Na^+, K^+-ATPase with mutant γ-subunit in insect Sf9 cells, normal routing of the α-subunit was disturbed as well, indicating that the mutation in the γ-subunit affects the complete ATPase complex (Meij *et al.* 2000b).

To investigate the dominant nature of the mutation, we measured serum Mg^{2+} concentration in two individuals with a heterozygous 11q23-ter deletion (including the *FXYD2* gene as determined by FISH analysis). Both individuals had normal serum Mg^{2+}-values, indicating that the loss of one *FXYD2* gene copy is not sufficient to cause a Mg^{2+}-deficient phenotype. This strongly suggests that the hypomagnesemia in our patients is caused by a dominant negative effect rather than haploinsufficiency (Meij *et al.* 2000b). Our current hypothesis on how this would influence tubular Mg^{2+}-reabsorption involves the putative Mg^{2+}-entry channel present in mouse distal convoluted tubule cells as described by Ritchie *et al.* (1996). When these cells were cultured under K^+ depleted conditions, apical entry of Mg^{2+} was blocked in these cells. We suggest that the mutation in the γ-subunit will also affect normal routing of the Na^+, K^+-ATPase complex to the plasma membrane, thus limiting the amount of K^+-entry into the cell via this pathway and disturbing normal ion homeostasis. Closing of the K^+-sensitive apical Mg^{2+}-channel would then cause reduced Mg^{2+}-reabsorption and hypomagnesemia (Figure 2). An alternative hypothetical model would be that cells expressing the mutant protein are more sensitive to induction of apoptosis. This would include the DCT where the putative apical Mg^{2+}-channel resides.

DCT cells may be particularly susceptible to alterations in ion homeostasis. The protein involved in Gitelman's syndrome, NCC, is also specifically expressed there. If NCC is blocked by thiazide, apoptosis of DCT cells is provoked as was shown in the rat (Loffing *et al.* 1996). Further, NCC knockout mice show apoptosis of DCT cells (Kaissling & Loffing 1998).

Renal hypomagnesemia, normocalciuria

A case of recessive renal hypomagnesemia (OMIM 248250), different from HHN, has been reported by Geven *et al.* (1987b). Two of four children of a second cousin consanguinous marriage were diagnosed with hypomagnesemia within the first year of life. Unlike other Mg^{2+}-wasting diseases, no abnormalities in Ca^{2+}-excretion or serum Ca^{2+}-concentration were detected. Intestinal Mg^{2+}-absorption studies were carried out with $^{28}Mg^{2+}$ in one of the two patients. Total body retention and resorption by the gut were elevated. However, despite the hypomagnesemia, urinary

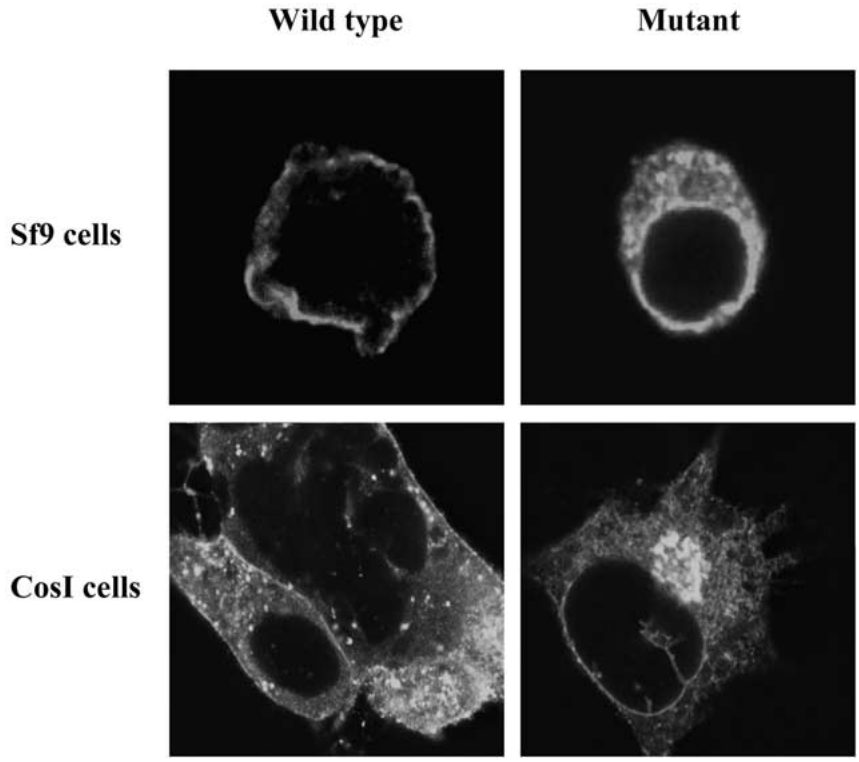

Figure 1. Na^+,K^+-ATPase γ-subunit routing defect observed in both insect Sf9 cells (upper panels) and mammalian COS1 cells (bottom panels), when comparing wild type (left) and mutant (right) expression (Meij *et al.* 2000).

Mg^{2+}-excretion remained in the normal range; in a properly functioning kidney urinary excretion should be very low. In this respect, this disease entity resembles dominant renal hypomagnesemia. To our knowledge, this is the only familial hypomagnesemia disease described in literature without associated disturbance in Ca^{2+}-homeostasis. With polymorphic markers, we investigated the currently known human hypomagnesemia loci on 11q23, 9q12–22.2 and 3q28–29 in this family. The exclusion of linkage with each of these regions (unpublished results) indicates that yet a fourth locus involved in Mg^{2+}-homeostasis remains to be identified.

Disorders associated with disturbed magnesium handling

Gitelman's syndrome

In Gitelman's syndrome (GS, OMIM 263800), hypomagnesemia is seen in combination with hypocalciuria, hypokalemia and metabolic alkalosis. Renin activity and aldosterone are elevated. In contrast to the more severe Bartter's syndrome (see below), GS is a relatively mild disease, reflected by the absence of growth retardation and polyuria and the relatively late age of onset (above 6 years of age). Patients often are asymptomatic, except for transient periods of weakness and tetany (Gitelman 1966). Chronic hypomagnesemia is assumed to underlie the chondrocalcinosis seen in a minority of adult patients (Smilde *et al.* 1994; Punzi *et al.* 1998).

GS is a recessive disorder, caused by mutations in the *SLC12A3* gene (OMIM 600968) cloned in 1996 (Simon *et al.* 1996a; Lemmink *et al.* 1996). It encodes the thiazide sensitive Na^+,Cl^--cotransporter, NCC and is expressed in the apical membrane of DCT cells (Obermueller *et al.* 1995). Since the identification of the gene, more than 100 different mutations have been reported with a possible clustering of mutations at the C-terminal end (Simon *et al.* 1996a; Mastroianni *et al.* 1996; Lemmink *et al.* 1996, 1998). By functional expression studies in *Xenopus* oocytes, it was shown that most of these mutations lead to defective processing and/or intracellular routing of the cotransporter (Kunchaparty *et al.* 1999).

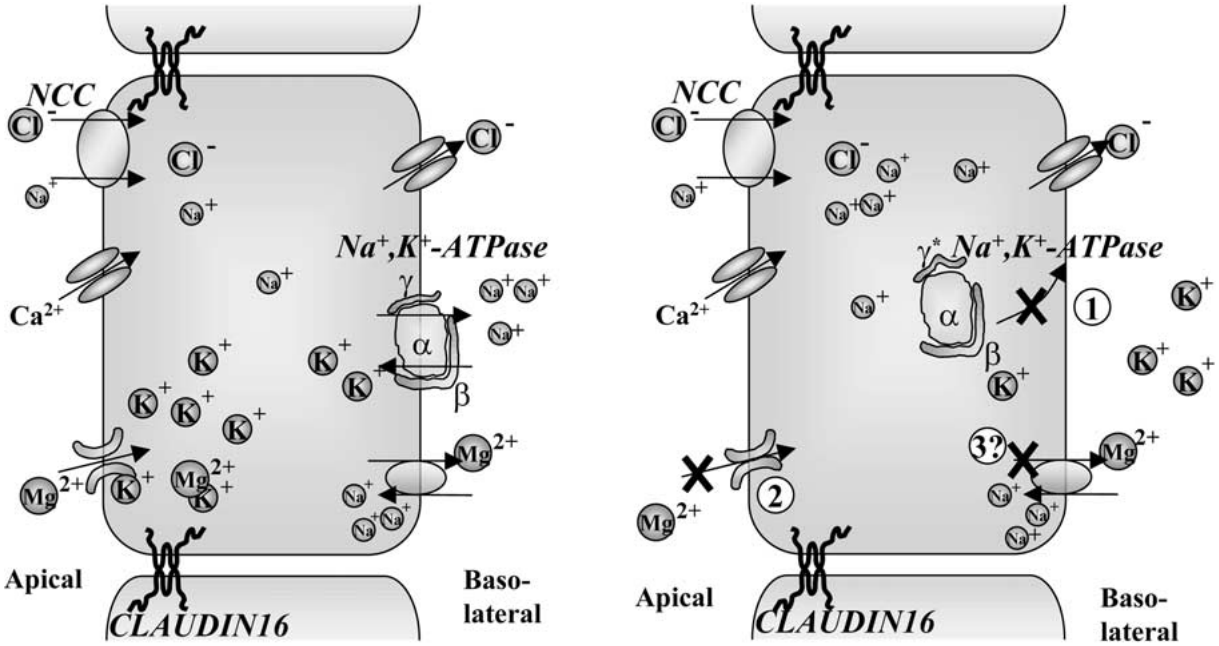

Figure 2. Hypothetical model of the involvement of the Na^+,K^+-ATPase γ-subunit in Mg^{2+} reabsorption by DCT cells including putative K^+-sensitive apical Mg^{2+}-entry and basolateral Mg^{2+}-extrusion mechanisms (Ritchie *et al.* 1996). *Left*: Wild type situation in which the apical Mg^{2+}-channel is open. *Right*: Putative effect of the Na^+,K^+-ATPase γ-subunit mutation involves disturbed routing of the Na^+, K^+-ATPase α β γ-complex (1) leading to a decreased intracellular K^+-concentration which closes the apical Mg^{2+}-channel (2). Additionally, reduced efflux of Na^+ and decreased availability of intracellular Mg^{2+} may also inhibit the basolateral Mg^{2+}-extrusion mechanism (3).

The disruption of Na^+,Cl^--cotransport in the DCT explains most of the features of GS. Because NaCl reabsorption is lowered in the DCT, more Na^+ will arrive at the connecting tubule and the cortical collecting duct (CCD) resulting in mild volume contraction. This stimulates the renin-angiotensin system, increasing renin and aldosterone. The subsequent increase of K^+ and H^+ secretion in exchange for Na^+ in the CCD gives rise to hypokalemia and metabolic alkalosis. The reduced influx of NaCl in the DCT cells in combination with continued basolateral Cl^- extrusion is assumed to cause hyperpolarization. This in turn induces an increase in Ca^{2+}-reabsorption, explaining the lowered excretion of Ca^{2+} (Friedman 1998).

It is still a matter of debate what causes the lowered serum magnesium levels in GS. Generally, hypomagnesemia is presumed to be secondary to the other features, although NCC knock-out mice show hypomagnesemia without hypokalemia (Schultheis *et al.* 1998). One hypothesis assumes that the disruption of NCC would affect the transmembrane potential in such a way that Mg^{2+} could passively leak away paracellularly through CLDN16 (Ellison 2000). Alternatively, there is evidence for both an apical Mg^{2+}-channel and a basolateral Mg^{2+} extrusion mechanism in DCT cells which could be affected by differences in NaCl homeostasis within the DCT cells (Ritchie *et al.* 1996). Finally, as noted before, there is evidence for apoptosis of DCT cells in NCC knock-out mice (Kaissling & Loffing 1998). Possibly, the death of DCT cells including the putative active Mg^{2+} transport system located here could cause the GS phenotype and concurrently explain the hypomagnesemia.

Bartter's syndrome

Bartter's syndrome (BS) was first described by Bartter *et al.* in 1962 (OMIM 601678, 602023). Its main features are hypokalemia and metabolic alkalosis, associated with normo- to hypercalciuria. Renin activity and aldosterone are elevated in serum, with normal blood pressure. In addition BS patients may also have hypercalciuria, nephrocalcinosis and elevated urinary prostaglandin E_2 secretion. In up to 30% of BS patients hypomagnesemia is also observed. Patients fail to thrive and show developmental delay. BS patients who do not present antenatally usually present in childhood before the age of 6 years, often as a failure to thrive (Guay-Woodford 1998; Schwarz & Alon

1996). The classification of BS is based on the four different genetic loci that have been implicated in BS to date.

Mutations found in *NKCC2* (OMIM 600839) cause type I BS which is characterized by severe hypokalemia (Simon *et al.* 1996b; Vargas-Poussou *et al.* 1998). *NKCC2* encodes the bumetanide sensitive Na^+, K^+, $2Cl^-$-cotransporter, localized to the apical membranes of medullary thick ascending limb (mTAL) cells. Type II BS is caused by mutations in the mTAL lumenal K^+-channel ROMK (OMIM 600359) which is also involved in the secretion of K^+ in the CCD (Simon *et al.* 1996c; ICSBS 1997). Type III BS is caused by mutations in the CLCNKB gene, encoding the basolateral Cl^--channel and also present in the mTAL (OMIM 602023) (Simon *et al.* 1997; Konrad *et al.* 2000). A fourth locus on chromosome 1p31 has been linked to BS with sensorineural deafness (OMIM 602522), but no disease gene has yet been identified (Landau *et al.* 1995; Brennan *et al.* 1998; Vollmer *et al.* 2000).

In all types of BS, the identified mutations lead to an increased distal load of NaCl causing a more severe hypokalemia and metabolic alkalosis than in GS since there is more severe volume contraction. The majority of BS patients with hypomagnesemia (up to 30%) were shown to have mutations in the CLCNKB gene (Type III BS) (Konrad *et al.* 2000). The precise mechanism responsible for Mg^{2+}-wasting in BS is as yet unknown.

Calcium sensing receptor disorders

The Ca^{2+}/Mg^{2+}-sensing receptor is expressed in the parathyroid gland and in the kidney, as well as a number of other tissues including thyroidal C-cells, brain and the gastrointestinal tract (reviewed by Hebert *et al.* 1997; Brown & MacLeod 2001). It responds to extracellular calcium concentration (Ca_0^{2+}) and mediates several of the known effects of Ca_0^{2+} on parathyroid and renal function. In adult rat kidney, the *CasR* gene is predominantly expressed in the basolateral membranes of the TAL and to a lesser extent in CCD and other nephron segments (Riccardi *et al.* 1995; Butters *et al.* 1997). It is believed both to sense Ca^{2+}- and Mg^{2+}-levels and regulate the reabsorption of these ions (Chattopadhyay *et al.* 1997) presumably via a G-protein coupled response. The function of CasR in the distal tubule is assumed to prevent saturation of Ca^{2+} and Mg^{2+} by adjusting water excretion through regulation of apical water channels (e.g., aquaporin 2), thereby preventing stone formation (Sands *et al.* 1997).

Several syndromes are associated with mutations in the CasR gene. Familial benign hypercalcemia was first described by Foley *et al.* in 1972. In addition, hypocalciuria and mild hypermagnesemia were found in patients with this syndrome (Law & Heath 1985; Marx *et al.* 1981). The *CasR* gene was identified as the underlying gene more than two decades later (Brown *et al.* 1993). Heterozygous mutations in the sensor gene were shown to cause hypocalciuria and hypercalcemia whereas patients with homozygous mutations develop severe neonatal hyperparathyroidism (Pollack *et al.* 1993; Pearce *et al.* 1995). This was confirmed in a knockout mouse model which displayed the same phenotype as the homozygous patients (Ho *et al.* 1995). Finally, gain of function mutations in the *CasR* gene cause autosomal dominant hypercalciuric hypocalcemia (Pollack *et al.* 1994; Pearce *et al.* 1996; Okazaki *et al.* 1999).

As for individuals with *CLCNKB* (Type III BS) mutations, not all individuals carrying mutations in the *CasR* gene present with a Mg^{2+}-phenotype. The disturbances in renal Mg^{2+}-handling that have been described are mild hypomagnesemia and are observed in almost 50% of the patients with gain of function mutations. Also, lowered excretion of Mg^{2+} and sometimes hypermagnesemia are associated with defective CASR (reviewed by Cole & Quamme 2000).

Concluding remarks and future outlook

In recent years, our knowledge of the genetics of Mg^{2+}-handling has greatly increased. The identification of the CLDN16 protein and the involvement of the Na^+, K^+-ATPase γ-subunit in renal Mg^{2+}-handling have changed our insights but have also raised new questions. What is the function of the CLDN16 protein in the DCT where the transepithelial voltage difference does not favour Mg^{2+}-reabsorption? Why do we not see more severe electrolyte disturbances in patients with a mutation in the Na^+, K^+-ATPase γ-subunit? Why do most patients with the *FXYD2* mutation not show a clinical phenotype? Similar questions can soon be asked about the gene responsible for HSH since the responsible gene has been mapped to within a 1 cM region.

In addition to these diseases that have been 'genetically' explained, there are still hereditary disorders of Mg^{2+}-homeostasis for which the causative gene

has not yet been identified. One example is the recessive renal hypomagnesemia described by Geven *et al.* (1987b). To date, identification of genes responsible for disturbances of Mg^{2+} homeostasis has relied on positional cloning approaches using family pedigrees as a guide. For syndromes not yet explained, candidate gene screening is becoming a very attractive and more feasible approach given a draft human genomic sequence and the advent of gene chips. For example, it might be interesting to investigate genes homologous to *CLDN16*. Kidney specific homologues like the *CLDN2* gene (Furuse *et al.* 1998) might be involved in other forms of renal Mg^{2+} wasting. Additionally other members of the *FXYD* gene family encoding ion transport inducing proteins might be candidates. Another interesting gene was mentioned by Ritchie *et al.* (1996). When cultured under Mg^{2+}-deficient conditions the so called Mg^{2+}-responsive element Mre was differentially expressed in mouse kidney cells. Although the function of this gene is not known yet, it might act as a sensor for Mg^{2+}, similar to the *CasR* gene.

Candidates can also arise from studies in species other than humans where interesting genes that are known or suspected to have a function in Mg^{2+}-handling have been identified. The *Mrs2p* gene encodes a membrane bound RNA splicing factor present in the yeast inner mitochondrial membrane with faint homology to the ubiquitous bacterial CorA Mg^{2+} transporter (Bui *et al.* 1999). Mutations in *Mrs2p* give a respiratory chain deficiency (*pet*$^-$ phenotype) and a decreased mitochondrial Mg^{2+} content. Bui *et al.* (1999) reported that overexpression of a bacterial *corA* gene could partially rescue the *Mrs2p* mutants, implying that the enzyme was also a Mg^{2+} transporter although no actual Mg^{2+} transport has been demonstrated to date. Recently, from yeast, the *Lpe10p* gene, a putative functional homologue of the *Mrs2p* gene was cloned (Gregan *et al.* 2001). Such candidate genes, either from the human genome or other species if they have a human homologue, would be excellent candidates for the as yet unexplained disorders of Mg-homeostasis. We're well on our way to a genetic explanation of many if not most Mg^{2+} disorders, information that will clearly provide both the physiological basis of the syndrome and hopefully new avenues for treatment.

Endnote

[1]OMIM refers to the "Online Mendelian Inheritance in Man" database maintained by the United States National Institutes of Health. Specific entries in this database can be accessed at the following URL: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM. Enter the OMIM number given in the text in the search window that appears.

Note added in proof

Very recently, Birkenhäger *et al.* (2001) identified 7 different mutations in the *BSND* gene in patients with BS and sensorineural deafness. The *BSND* gene is expressed in the thin and thick ascending limb of Henle's loop in the kidney and in the dark cells of the inner ear. It encodes the BARTTIN protein, which functions as a γ-subunit for the basolateral chloride channels CLCNKA and CLCNKB and is crucial for renal Cl^- reabsorption and inner ear K^+ secretion (Estévez *et al.* 2001).

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