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R E V I E W

Hereditary tubular transport disorders: implications for renal handling of Ca^{2+} and Mg^{2+}

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A B S T R A C T

The kidney plays an important role in maintaining the systemic Ca^{2+} and Mg^{2+} balance. Thus the renal reabsorptive capacity of these cations can be amended to adapt to disturbances in plasma Ca^{2+} and Mg^{2+} concentrations. The reabsorption of Ca^{2+} and Mg^{2+} is driven by transport of other electrolytes, sometimes through selective channels and often supported by hormonal stimuli. It is, therefore, not surprising that monogenic disorders affecting such renal processes may impose a shift in, or even completely blunt, the reabsorptive capacity of these divalent cations within the kidney. Accordingly, in Dent's disease, a disorder with defective proximal tubular transport, hypercalciuria is frequently observed. Dysfunctional thick ascending limb transport in Bartter's syndrome, familial hypomagnesaemia with hypercalciuria and nephrocalcinosis, and diseases associated with Ca^{2+}-sensing receptor defects, markedly change tubular transport of Ca^{2+} and Mg^{2+}. In the distal convolutions, several proteins involved in Mg^{2+} transport have been identified [TRPM6 (transient receptor potential melastatin 6), proEGF (pro-epidermal growth factor) and FXYD2 (Na^+/K^+-ATPase γ-subunit)]. In addition, conditions such as Gitelman's syndrome, distal renal tubular acidosis and pseudohypoaldosteronism type II, as well as a mitochondrial defect associated with hypomagnesaemia, all change the renal handling of divalent cations. These hereditary disorders have, in many cases, substantially increased our understanding of the complex transport processes in the kidney and their contribution to the regulation of overall Ca^{2+} and Mg^{2+} balance.

INTRODUCTION

Divalent cations are essential for physiological processes, such as intracellular signalling, enzyme activation, neural excitability, muscle contraction and bone formation. Overall Ca^{2+} balance is regulated via a co-ordinated interplay between the parathyroid glands, bone, intestine and kidneys. Systemic Mg^{2+} concentrations are

Key words: divalent cation, kidney, hereditary disorder, reabsorption, tubular transport.

Abbreviations: BP, blood pressure; BSND, Bartter's syndrome with sensorineural deafness; CaSR, Ca^{2+}-sensing receptor; CD, collecting duct; CLC, Cl^- channel; CNT, connecting tubule; DCT, distal convoluted tubule; dRTA, distal renal tubular acidosis; EGF, epidermal growth factor; EGFR, EGF receptor; ENaC, epithelial Na^+ channel; FHH, familial hypocalciuric hypercalcaemia; FHHNC, familial hypomagnesaemia with hypercalciuria and nephrocalcinosis; FXYD2, Na^+/K^+-ATPase γ-subunit; GFR, glomerular filtration rate; HSH, hypomagnesaemia with secondary hypocalcaemia; IRH, isolated autosomal-recessive hypomagnesaemia; NCC, Na^+–Cl^- co-transporter; NHE3, Na^+/H^+ exhanger 3; NKCC2, furosemide-sensitive Na^+–K^+–$2Cl^-$ co-transporter; NSHPT, neonatal severe primary hyperparathyroidism; OCRL, oculocerebrorenal syndrome of Lowe; PCT, proximal convoluted tubule; PHAII, pseudohypoaldosteronism type II; PST, proximal straight tubule; PTH, parathyroid hormone; ROMK, renal outer medullary K^+ channel; TAL, thick ascending limb; TRP, transient receptor potential; TRPM, TRP melastin; TRPV, TRP vanilloid; WNK kinase, with-no-lysine kinase; ZO-1, zona occludens-1.

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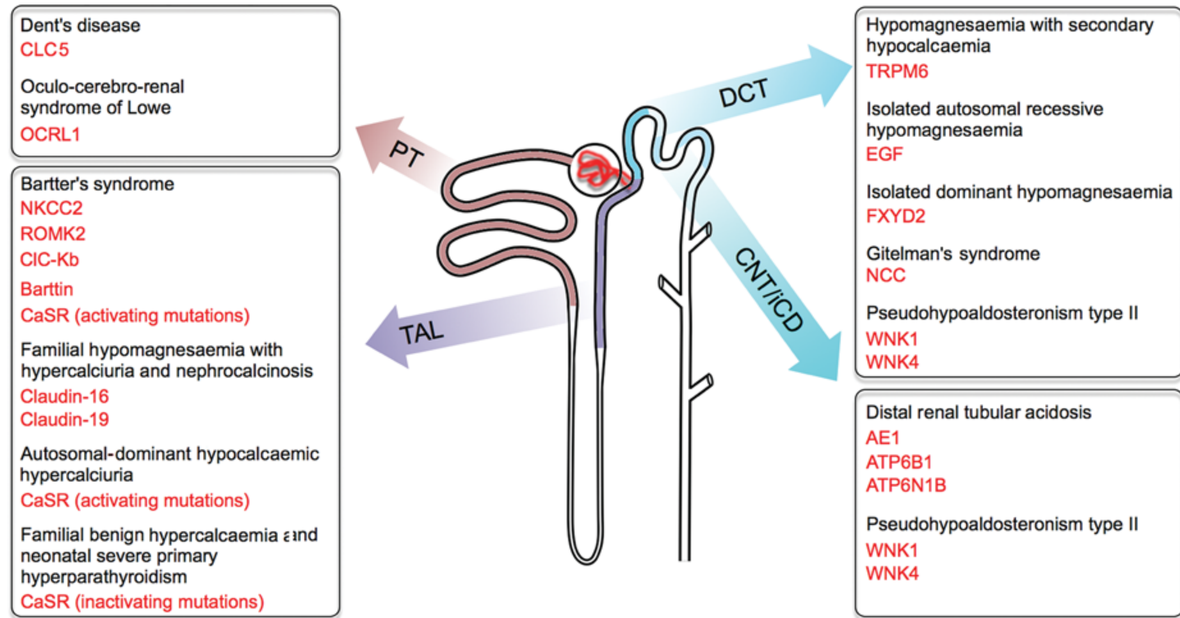

Figure 1 Schematic representation of the nephron listing the predominant origins of intrarenal transport defects causing dysfunctional renal handling of divalent cations

In most species, the transition from the DCT to the CNT is gradual, whereas the transition between these nephron segments in the rabbit is very abrupt. In species where these borders are less well defined with larger segments containing DCT and CNT cells intermingled, the occurrence of the CNT cell is often considered the start of CNT segment [108]. In addition to the CNT cells, intercalated cells involved in acid/base secretion also arise in this segment [108]. Note that defects causing PHAII probably originate from both the DCT and CNT segments, whereas dRTA is caused by distrubed acid/base handling in intercalated cells of the CNT and CD regions. iCD, initial CD; PT, proximal tubules.

maintained in a similar manner; however, the exact mechanisms governing plasma Mg^{2+} concentrations are less well defined. As essential cellular functions are reliant on maintaining extracellular Ca^{2+} and Mg^{2+} concentrations within a narrow range, the consequences of disturbances in these divalent cations often result in symptoms related to instability of the neurological and cardiac systems. Hence hypercalcaemia can lead to fatigue, nausea, coma and abnormal heart rhythms that can result in cardiac arrest [1]. Hypocalcaemia can produce tetany, carpopedal spasms and life-threatening arrhythmias [2]. Hypermagnesaemia also causes severe symptoms, including lethargy, coma and cardiac arrest [3]. Similarly, hypomagnesaemia promotes neuromuscular irritability, such as tetany and seizures, as well as cardiac arrhythmias. Secondary hypocalcaemia is also commonly observed in patients with hypomagnesaemia, further complicating their condition [3].

The kidney plays a central role in controlling the excretion of Ca^{2+} and Mg^{2+} in response to conditions of deprivation or excess of these cations [4,5]. Transepithelial (i.e. net result of paracellular and transcellular) transport of divalent cations across the epithelia differs in the various nephron segments. Accordingly, the proximal tubule and TAL (thick ascending limb) reabsorb these ions in a passive paracellular manner, whereas in the distal convolutions active transcellular transport occurs. Many inherited renal diseases related to electrolyte and even protein transport cause defects in the handling of Ca^{2+} and Mg^{2+} by the kidney (Figure 1). The aim of the present review is to summarize the current knowledge pertaining to these diseases and how they have contributed to our understanding of renal divalent cation handling.

PROXIMAL TUBULES

Plasma free/ionized Ca^{2+} levels are maintained within a narrow range around the set-point value of 1.2 mmol/l [6]. Approx. 50–60 % of total plasma Ca^{2+} is freely diffusible, the majority as ionized Ca^{2+}, the remaining complexed to other ions. This free portion of the total Ca^{2+} is filtered by the glomeruli. The remainder is bound to plasma proteins, predominantly albumin, and is largely retained in plasma [7–9]. Of the filtered Ca^{2+}, only 1–2 % is excreted by the kidney, the largest fraction being reclaimed by the renal tubules [7,9]. Detailed micropuncture studies have estimated that approx. 55–60 % of the filtered Ca^{2+} is re-absorbed along the PCT (proximal convoluted tubule). An additional 10 % is reabsorbed in the PST (proximal straight tubule) [10–13].

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Ca^{2+} reabsorption in the PCT occurs in proportion to Na^+ and water. Hence proximal tubular Ca^{2+} transport is thought to be primarily passive in nature [14]. The tubule fluid/filterable plasma Ca^{2+} ratio has been reported to range from 1.0 to 1.2 along the PCT [11,15]. Thus the isotonic removal of Na^+ and water from the lumen increases the intraluminal Ca^+ concentration and facilitates secondary Ca^{2+} reabsorption [11,12]. This is in line with the existence of a paracellular shunt pathway, where the electrochemical gradient across the epithelia sets the direction of transport. Importantly, volume expansion (probably by reducing proximal Na^+ and water reabsorption and, hence, the electrochemical driving force for Ca^{2+}) has been demonstrated to reduce proximal tubular Ca^{2+} reabsorption, whereas volume contraction increases the proximal tubular reabsorptive capacity [16].

The systemic Mg^{2+} concentration is maintained between 0.75 and 1.0 mmol/l [17]. Approx. 70 % of Mg^{2+} in serum is freely filterable, either existing as ionized free Mg^{2+} or complexed with filterable anions [18]. Thus the concentration of Mg^{2+} reached in the initial glomerular filtrate is approx. 0.5 mmol/l [8,19]. The kidney excretes approx. 3–5 % of the Mg^{2+} found in the ultrafiltrate [20]. Although the PCT has a high permeability to Na^+ and Ca^{2+}, as well as a high tubular reabsorptive capacity for these ions, a limited amount of Mg^{2+} (approx. 10–20 %) is reabsorbed in this nephron segment [15,19,21]. Similar permeability properties have been reported for the PST [22]. Thus the entire proximal tubule is responsible for reclaiming some 25 % of the filtered Mg^{2+}. The short half-life of the radioactive $^{28}Mg^{2+}$ isotope makes it unsuitable as a tracer and has seriously hampered the experimental determination of Mg^{2+} fluxes and permeability coefficients. Hence most determinations of Mg^{2+} in tubular punctates have been performed using electron probes, limiting the determinations to net Mg^{2+} fluxes. Under these experimental settings, proximal tubular Mg^{2+} transport is generally thought to be paracellular. Owing to the apparent permeability differences, Na^+-driven water transport increases the intraluminal concentration of Mg^{2+} along the proximal tubular epithelium [22,23]. It has been determined that a tubule fluid/filterable Mg^{2+} ratio of 1.9 is necessary for Mg^{2+} reabsorption to occur in the segment [15]. This suggests that adequate water removal is a prerequisite for Mg^{2+} transport to occur and may, therefore, only commence in the late PCT, when the luminal Mg^{2+} concentration is sufficiently high [15]. Moreover, the high concentration gradient necessary for transepithelial Mg^{2+} transport suggests a poor permeability of the tight junctions to Mg^{2+} in the proximal tubule. Interestingly, the proximal reabsorptive capacity in the neonatal rat is much higher, as reabsorption of filtered Mg^{2+} ranges between 60 and 70 % [24]. Whether this represents a remodelling of the selectivity filter in the tight junction protein complex during development remains unclear. In contrast with Ca^{2+}, volume expansion does not appear to affect proximal tubular Mg^{2+} reabsorption [25].

TRANSPORT DEFECTS OF THE PROXIMAL TUBULE AFFECTING DIVALENT CATION HANDLING

Dent's disease

Dent's disease is a recessive X-linked proximal tubular disorder associated with low-molecular-mass proteinuria. Males usually present with a more severe phenotype, including aminoaciduria, hypercalciuria, nephrolithiasis, glycosuria and progressive renal failure [26–28]. Individuals with Dent's disease typically have normal plasma Mg^{2+} concentrations. Some patients present with rickets, which is normalized after pharmacological 1,25-dihydroxyvitamin D_3 treatment [26]. In general, patients with Dent's disease have mildly elevated 1,25-dihydroxyvitamin D_3 levels [26,29]. Thus 1,25-dihydroxyvitamin D_3 may serve to stabilize plasma Ca^{2+} levels secondary to renal Ca^{2+} wasting or elevated 1,25-dihydroxyvitamin D_3 levels may by itself augment the hypercalciuria [29].

Dent's disease is caused by mutations in the *CLC5* gene [28]. CLC5, originally identified as a Cl^- channel, is also known to function as a lysosomal Cl^-/H^+ antiporter involved in endosomal acidification [30,31]. CLC5 localizes to the proximal tubule, the TAL and the CD (collecting duct) intercalated cells [32]. Genetic ablation of *Clc5* in mice impairs proximal tubular endocytosis of proteins by inhibiting the trafficking of megalin and cubilin [33–35], largely explaining the low-molecular-mass proteinuria. Similarly, aminoaciduria and glycosuria may occur due to defective trafficking of the transport proteins responsible for proximal tubular reabsorption of these solutes [36]. It is important to note that two different mice strains have been generated with a targeted deletion of *Clc5* [34,35]. Although many of the renal phenotypic characteristics observed in Dent's disease are present in both mice, important differences are observed in terms of hypercalciuria, 1,25-dihydroxyvitamin D_3 levels, nephrocalcinosis and renal failure (reviewed in detail in [37]). Thus, currently, the exact underlying mechanism responsible for hypercalciuria remains to be clarified.

Interestingly, a kidney-specific deletion of megalin (*Lrp2*) in mice causes hypocalcaemic hypercalciuria, vitamin D deficiency and extrarenal symptoms such as rickets [38,39]. The inactive vitamin D precursor 25-dihydroxyvitamin D_3, complexed to the vitamin D-binding proteins, has been shown to be reabsorbed in the proximal tubules by the endocytic receptor megalin [39]. Thus megalin-mediated uptake of inactive 25-dihydroxyvitamin D_3 by the proximal tubule appears a

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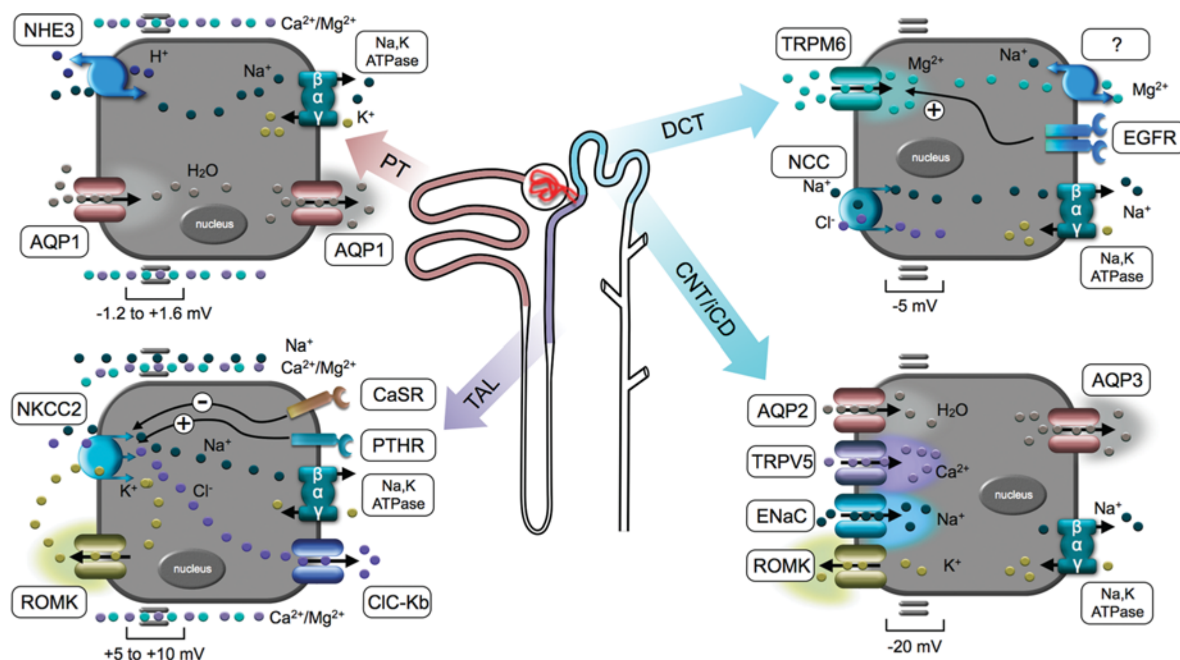

Figure 2 Schematic representation of the cellular composition of renal ion and water transport proteins within the various nephron segments

The iCD (intial CD) represents the start of the CD principal cells arising in the late distal convolutions. The CNT and the iCD principal cells express TRPV5, as does NCC-expressing cells in the DCT2 segment in many species [118–121]. The DCT cell portrayed in the Figure resembles one located in the early DCT region. Transepithelial voltages are listed for the PT [204], TAL [55,56], although the transepithelial voltage may increase to approx. +30 mV [57–59], DCT [55,10,111] and CNT [109]. AQP, aquaporin; PTHR, PTH receptor; ATP6B1 (ATP6V1B1), B1 subunit of H^+ ATPase; ATP6N1B (ATP6V0A4), 116 kDa subunit a isoform 4 of H^+ ATPase; PT, proximal tubules.

prerequisite for conversion into its active form 1,25-dihydroxyvitamin D_3. However, it is currently unknown whether this mechanism contributes to hypercalciuria in Dent's disease, as impaired uptake is not reflected in the serum 1,25-dihydroxyvitamin D_3 concentrations. Whether ablation of the transporter in more distal segments promotes hypercalciuria remains untested.

OCRL (oculocerebrorenal syndrome of Lowe)

OCRL is an X-linked disease caused by mutations in *OCRL1*, which encodes an inositol polyphosphate 5-phosphatase [40]. Affected males have congenital cataracts, mental retardation and renal tubular dysfunction. Evidence suggests that OCRL1 protein deficiency can impair lysosomal trafficking in the proximal tubule via accumulation of the substrate phosphatidylinositol 4,5-bisphosphate [41,42]. Thus, similar to Dent's disease, massive low-molecular-mass proteinuria as well as other characteristics of proximal tubular dysfunction are observed. Additionally, renal tubular acidosis is often detected in patients with Lowe's syndrome. Individuals suffering from the syndrome only infrequently display normocalcaemic hypercalciuria and calcifications [43]. Strikingly, patients have also been identified with mutations in *OCRL1* that develop an isolated renal Dent's disease-like phenotype with hypercalciuria, but without tubular acidosis and extrarenal symptoms, such as cataracts and neurological abnormalities [44]. It is currently unknown how mutations in a single gene can cause two supposedly distinct phenotypes.

TAL

Micropuncture experiments have demonstrated that approx. 20–25 % of the filtered Ca^{2+} and 60 % of filtered Mg^{2+} is reabsorbed in the TAL [10,11,45]. In this segment, the transport of divalent cations occurs in a passive paracellular manner. In most species, the cortical TAL appears to be most important for paracellular transport, as medullary transport is negligible under basal conditions [46–49]. The lumen-positive transepithelial voltage appears to be the most important driving force for Ca^{2+} and Mg^{2+} reabsorption. Furosemide, a potent inhibitor of Na^+, K^+ and Cl^- co-transport, almost completely abolishes the transepithelial voltage gradient across the TAL [50–52]. Under these conditions, Ca^{2+} and Mg^{2+} transport is drastically inhibited [53,54]. As shown in Figure 2, NKCC2 (furosemide-sensitive Na^+–K^+–$2Cl^-$ co-transporter) serves as the apical entry mechanism

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for Na^+, K^+ and Cl^- in the TAL. Similarly, ROMK2 (renal outer medullary K^+ channel 2) is necessary for apical K^+ recycling across the apical membrane. Inwardly transported Cl^- exits across the basolateral membrane, predominantly via CLC-Kb channels. The Na^+/K^+-ATPase constitutes the initial driving force for Na^+ transport in the TAL. Other transporters involved in vectorial Na^+ transport have also been identified [i.e. NHE3 (Na^+/H^+ exhanger 3)] in the medullary TAL. However, their exact contribution to overall Na^+ transport within the loop is unclear.

The generation of a lumen-positive transepithelial voltage drives the paracellular reabsorption of Ca^{2+} and Mg^{2+} from the lumen. The electric gradient is likely to be made up of two mechanisms. NKCC2-mediated uptake of Na^+, K^+ and two Cl^- ions coupled with the subsequent secretion of K^+ across the apical membrane generates a lumen-positive voltage of approx. 5–10 mV [55,56]. Secondly, reabsorption of Na^+ along the length of the TAL reduces the intraluminal Na^+ concentration. Under these conditions, backflux of Na^+ from the interstitium to the tubular lumen may further augment voltage to values of approx. 30 mV [57–60].

cAMP-elevating hormones such as PTH (parathyroid hormone) stimulate Na^+/Cl^- transport in the TAL, probably by modulating these transport processes [49,61]. Interestingly, after application of PTH, changes in net Ca^{2+} and Mg^{2+} transport appear much higher than the concomitant changes reported in the transepithelial voltage. These observations suggest that alterations in the permeability of the paracellular pathway can modulate transepithelial divalent cation transport [61]. Similarly, CaSR (Ca^{2+}-sensing receptor) is expressed in the basolateral membrane of the TAL, where it modulates transport in response to changes in extracellular divalent cations [62,63]. Here activation of CaSR can inhibit vasopressin-induced cAMP formation by as much as 90 % [64]. Conversely, *in vitro* microperfusion experiments have shown that activation of the receptor inhibits net transport of Ca^{2+}, while leaving the transepithelial voltage and net Na^+/Cl^- flux intact [64,65]. However, chronic stimulation of the receptor is likely to reduce NKCC2 abundance as its expression is stimulated by cAMP. This is observed in rats administered gentamycin, an aminoglycoside antibiotic that activates CaSR [66,67]. In contrast, some reports have suggested that pharmacological inhibition of transepithelial voltage does not substantially affect Ca^{2+} transport in the TAL, which is suggestive of a transcellular pathway [48,65,68]. The nature of this transcellular route remains poorly defined, although it can presently not be excluded. Why only 20–25 % of the filtered Ca^{2+} is reabsorbed in the TAL, whereas as much as 60 % of filtered Mg^{2+}, is not entirely clear, but may represent differences in either delivery of divalent cations to the bend of Henle's loop or permeability differences for divalent cations within the TAL.

TRANSPORT DEFECTS OF THE TAL AFFECTING DIVALENT CATION HANDLING

Bartter's syndrome

Bartter's syndrome is characterized by severe renal Na^+/Cl^- wasting, hypokalaemic metabolic alkalosis, hyperreninaemia, hyperaldosteronism and prostaglandin E_2 hypersecretion, and patients present with a normo- or hypo-tensive phenotype [69–71]. Most gene defects causing Bartter's syndrome are inherited in an autosomal-recessive manner. Mutations in the genes encoding NKCC2 (*SLC12A1*) or ROMK (*KCNJ1*) lead to antenatal Bartter's syndrome [72,73]. Defects in either protein severely impair transport function in the TAL, produce renal Na^+/Cl^- wasting and dilute the interstitial gradient, resulting in a pronounced urinary concentrating defect. Thus antenatal Bartter's syndrome is the most severe form of Bartter's syndrome, being evident prenatally with polyhydramnios and often premature birth. Post-natally, severe polyuria causes volume depletion/dehydration and fever. Marked hypercalciuria, secondary nephrocalcinosis and osteopenia are hallmark features of antenatal Bartter's syndrome [71]. Interestingly, hypermagnesuria and hypomagnesaemia are not common characteristics of this type of Bartter's syndrome. CLC-Kb is a basolateral Cl^- channel located in the TAL, as well as more distal nephron segments [74]. In comparison with the apical entry and recycling mechanisms, CLC-Kb appears not to be the sole efflux pathway for Cl^- in the TAL. Accordingly, the K^+–Cl^- co-transporter and potentially other Cl^- channels located in the TAL may participate in removing intracellular Cl^- [75,76]. As suggested by Konrad et al. [75], the level of compensation provided by these efflux pathways may explain why the clinical definition is highly variable in patients with recessive *CLCNKB* (the gene encoding CLC-Kb) defects [75]. Thus individuals with CLC-K dysfunction and, hence, classical Bartter's syndrome can present with an antenatal Bartter's-syndrome-like phenotype to being almost asymptomatic [75,77]. In contrast with patients with antenatal Bartter's syndrome, hypercalciuria and nephrocalcinosis are an uncommon feature in these individuals; however, hypomagnesaemia occurs in up to 50 % of affected individuals [75,77]. Recessive mutations in *BSND* (Bartter's syndrome with sensorineural deafness; encoding Barttin, a β-subunit necessary for CLC-Kb function) lead to perhaps the most severe form of antenatal Bartter's syndrome, with sensorineural deafness and associated chronic renal failure [78,79]. In line with this, defects in both CLC-Ka and CLC-Kb confer a phenotype identical with that reported for *BSND* Barttin) mutations [80]. In contrast with CLC-Kb defects, mutations in *BSND* do not result in hypomagnesaemia. The absence of renal Mg^{2+} wasting has been attributed to the low GFR (glomerular filtration rate) often observed in BSND, paralleling the decline in

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renal function. Nephrocalcinosis is not a common feature of this disease [81].

Autosomal-dominant hypocalcaemic hypercalciuria is observed in patients with activating mutations in *CASR* (the gene encoding CaSR) [82,83]. Patients generally present with mild asymptomatic hypocalcaemia, and with serum Mg^{2+} and PTH levels in the lower-to-normal range [82]. Functional analysis of these mutant receptors revealed a left-shift in the dose–response curve; thus lower Ca^{2+} concentrations increase half-maximal activity of the receptor [82,83]. Some activating mutations appear to be more severe in changing the responses to extracellular Ca^{2+} and, thus, lead to an autosomal-dominant form of Bartter's syndrome [62,83,84]. These patients present with a phenotype similar to classical Bartter's syndrome, but with hypocalcaemia, suppressed secretion of PTH, renal Ca^{2+} wasting and nephrocalcinosis. Hypomagnesaemia and hypermagnesuria have also been reported in these patients [62,84].

It is interesting to note that, although all of the Bartter's syndrome types described are caused by inhibition of TAL transport and full or partial dissipation of the electrochemical gradient, there is a considerable variability in the consequence for the handling of divalent cations. Several theories have been presented to explain this diversity; however, the exact reasons for these controversies still remain largely enigmatic. Less Na^{+} loss is reported in most patients with Bartter's syndrome with CLC-Kb defects (in comparison with antenatal Bartter's syndrome), which often show a less severe phenotype and also a decreased prevalence of hypercalciuria and secondary nephrocalcinosis. It is difficult to directly envision the electrochemical gradients for Mg^{2+} and Ca^{2+} in the TAL of patients with Bartter's syndrome, as both volume depletion and polyuria will affect both systemic and tubular concentrations, and probably provoke compensatory mechanisms. However, it is probable that renal Na^{+} wasting is less severe in patients with Bartter's syndrome with CLC-Kb defects and, therefore, that the lumen-positive voltage is less affected. This may explain why these patients rarely present with hypercalciuria compared with individuals with antenatal Bartter's syndrome. Unfortunately, no clear correlation between urinary Na^{+} and Ca^{+} wasting has been presented in individuals with Bartter's syndrome. Thus additional phenotypic characterization of factors affecting Ca^{2+} wasting in Bartter's syndrome might elucidate this.

Why the opposite relationship is observed for renal Mg^{2+} handling between the Bartter's syndromes is also unclear. The low prevalence of hypermagnesuria in antenatal Bartter's syndrome suggests an enormous compensatory capacity of the DCT (distal convoluted tubule). In addition, patients with mutations in *CLCNKB* have a higher prevalence of hypermagnesuria. The expression of CLC-Kb has been reported to be the highest in the DCT [74]. Some patients with *CLCNKB* mutations present with a mixed Bartter's syndrome/Gitelman's syndrome phenotype with hypomagnesaemia [85]. Hence loss of CLC-Kb in segments distal to the TAL may contribute to this phenotype. The potential mechanism whereby this occurs is not known.

FHHNC (familial hypomagnesaemia with hypercalciuria and nephrocalcinosis)

Patients with the autosomal-recessive disorder FHHNC often develop severe hypomagnesaemia, due to a grossly increased fractional renal excretion of Mg^{2+}. Similarly, affected individuals have hypercalciuria, although systemic concentrations of Ca^{2+} are within the normal range [86,87]. Bilateral nephrocalcinosis is observed in all patients, and the severity of this finding is correlated with the progression of renal insufficiency. Ultimately, this leads to chronic renal failure. The primary problem is renal in origin, which is supported by the observation that affected individuals after renal transplantation normalize their plasma Mg^{2+} concentrations and return to a normocalciuric state [87].

Originally identified by Simon et al. [88], a defect in *CLDN16*, the gene encoding the tight junction protein claudin-16 (also known as paracellin-1), is now known to be the underlying cause of FHHNC [88]. Initial hydrophobic cluster analysis indicated that claudin-16 forms four transmembrane domains with intracellular C- and N-termini. The protein was found to localize to the tight junctions of the TAL, as well as the DCT [88–90], suggesting a possible role for claudin-16 in regulating the paracellular permeability to divalent cations. The mutations described often encode a single amino acid substitution in the transmembrane or extracellular domains of claudin-16, probably rendering the protein non-functional [88,91]. Another mutation in *CLDN16* has been reported, which confers a less severe phenotype with regression of the hypercalciuria and maintenance of the GFR. The mutation, which is found within a putative PDZ domain, appears predominantly to prevent the binding of claudin-16 to ZO-1 (zona occludens-1) and causes mislocalization of the protein to lysosomes [92]. Although the regulation of claudin-16 remains incompletely understood, phosphorylation of claudin-16 at Ser^{217} via the cAMP/PKA (protein kinase A) pathway appears to confer regulation by localizing claudin-16 to the tight junctions [93]. This is to some degree compatible with the observation that the cAMP-elevating hormone PTH can change the permeability of the paracellular pathway to Ca^{2+} and Mg^{2+} [61].

Individuals with a similar phenotype to patients with mutations in *CLDN16* have been described [89]. Affected individuals had severe hypomagnesaemia due to renal Mg^{2+} losses, in addition

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to bilateral nephrocalcinosis and renal insufficiency. The disease in these individuals was complicated by severe ocular problems, contrasting with infrequent milder ocular problems observed in patients with *CLDN16* mutations. Patients were found to have mutations in the *CLDN19* gene, which encodes claudin-19 in the same family of tight junction proteins. Complete co-localization with claudin-16 was found within the kidney [89], implicating a role in divalent ion homoeostasis.

Expressing claudin-16 in cells has generated highly variable results in terms of cation permeability (reviewed by [94]). When claudin-16 is expressed in LLC-PK1 cells, a high permeability for Na^+ is observed, while the Cl^- flux remains unaffected. Moreover, the permeability for Mg^{2+} was much smaller than that observed for Na^+ [59]. In the same cells, claudin-19 expression decreased absolute permeability to Cl^-, without affecting the Na^+ permeability [95]. When co-expressed, the tight junction becomes selective for cations [95]. This is in line with studies reporting a paracellular shunt pathway for Na^+ in the cortical TAL that is cation selective [57,96]. Expression of FHHNC-causing mutants of claudin-16 reduced selectivity for cations in LLC-PK1 cells [59]. Similarly, mutations in claudin-19 decreased its ability to block Cl^- permeation, leaving Na^+ permeability intact [95]. It remains unclear whether Mg^{2+} even permeates these claudins in sufficient degree to support the massive reabsorptive capacity for this ion in the TAL [59,94]. Using RNA interference, depletion of claudin-16 in mice also confers an FHHNC-like phenotype. Interestingly, microperfusion studies demonstrated that these mice have a 50 % reduction in the paracellular cation selectivity of the TAL (P_{Na}/P_{Cl}) [97]. Furthermore, no change in the selectivity between Na^+ and Mg^{2+} was found (P_{Na}/P_{Mg}), suggesting that claudin-16 functions predominantly as a non-selective cation channel [97].

How can changes in paracellular Na^+ fluxes explain the aetiology of FHHNC? On the basis of studies by Hou, Goodenough and co-workers [59,95], it is suggested that mutations in claudin-16 or claudin-19 in FHHNC would increase the permeability of the tight junction to anions, promote backflux of proportionally more Cl^-, and thereby dissipate the transepithelial voltage gradient. One would also expect some sort of Na^+/Cl^- wasting to occur in the TAL; however, patients with FHHNC due to a defective *CLDN16* gene retain their ability to conserve urinary Na^+ after Na^+/Cl^- deprivation, have normal aldosterone levels and have appropriate natriuretic responses to furosemide. Yet, their calciuretic and magnesiuretic response to furosemide is severely blunted [98]. Conversely, in the *Cldn16*-depleted mice, lowered BP (blood pressure), an increase in the urinary Na^+/K^+ ratio and elevated aldosterone levels are observed, along with enhanced CD Na^+ transport [97,99].

Familial benign hypercalcaemia and NSHPT (neonatal severe primary hyperparathyroidism)

Inactivating mutations in *CASR* have been identified in hypercalcaemic patients with FHH (familial hypocalciuric hypercalcaemia) and a severe neonatal form of primary hyperparathyroidism (NSHPT). Although FHH is inherited in an autosomal-dominant fashion, NSHPT is caused by homozygous inactivating mutations in *CASR*. Patients with FHH can have hypercalcaemia and hypermagnesaemia, while an inappropriate reduction in urinary Ca^{2+} excretion is observed. A lower renal excretion of Mg^{2+} has also been reported in FHH [100]. Moreover, serum PTH concentrations are often found within the normal range, in the presence of hypercalcaemia, or even elevated levels have been reported in a subset of patients [101]. Thus inactivating mutations in the *CASR* allele shifts the set point of Ca^{2+} in this condition, blunting the normal ability to reduce PTH secretion in response to elevated Ca^{2+} levels and by directly increasing renal Ca^{2+} and Mg^{2+} reabsorption [63,102–104]. Although patients with FHH can have lifelong hypercalcaemia and hypermagnesaemia, they are generally asymptomatic [104]. In NSHPT, homozygous inactivation of the *CASR* gene cause infantile hyperparathyroidism. This rare disorder is life-threatening, with severe hypocalciuric hypercalcaemia. Many extrarenal symptoms associated with hypercalcaemia are observed. In addition, polyuria and dehydration is commonly reported [63,105]. Renal hyperabsorption of divalent cations is partly caused by increased PTH levels, resulting from reduced suppression of PTH secretion in the CaSR-impaired parathyroid. In addition, an intrinsic mechanism due to reduced functionality or an absence of CaSR in the TAL occurs independently of PTH [106]. This becomes evident in *Casr*-deficient mice, as their lethal phenotype normally observed after deletion of the *Casr* gene can be rescued by crossing these mice with mice deficient in *Pth*. However, in these double-knockout mice with a complete absence of circulating PTH, the variance of serum Ca^{2+}, as well as the fractional excretion of Ca^{2+}, exceeded the variance observed in *Pth*-deficient mice with an intact CaSR [107]. These results suggest that CaSR is important in regulating Ca^{2+} homoeostasis also independently of regulating PTH secretion.

DCT, CNT (CONNECTING TUBULE) AND INITIAL CDs

The distal convolutions are often defined as comprising the DCT, CNT and the initial CD [108]. Several species differences can be observed in the composition and borders of the individual nephron segments, particularly between rabbit compared with human, mice

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and rat. Although the transition between the different nephron segments ends abruptly in the rabbit, the borders between these segments are more intermingled in the other species. Lumen negative transepithelial voltage increases from approx. −5 mV in the early convolutions up to approx. −40 mV or more towards the end of the late convolutions [109–113]. However, some variability in the measured voltages have been reported [109]. Detailed micropuncture studies have estimated that approx. 3–7 % of the filtered Ca^{2+} is reclaimed between the early or late distal sites and the final urine in the rat [10,19,114]. However, the absence of direct determinations of delivery in juxtamedullary nephrons may only provide approximate estimates. It is unclear whether these punctures represent the whole Ca^{2+}-transporting segment in the distal convolutions. Approx. 2 % of the filtered load is reabsorbed between early and late distal collections in the same nephron, whereas the amount doubles in the presence of PTH [115]. Ca^{2+} reabsorption is essentially thought to be active, as it occurs in the presence of lower intraluminal Ca^{2+} concentrations and lumen negative transepithelial voltages [10].

The TRPV5 [TRP (transient receptor potential) vanilloid 5] channel provides the main apical entry step for Ca^{2+} in the distal convolutions. TRPV5 was originally cloned from primary cultures of rabbit renal CNT/cortical CD tubules [116]. The channel localizes primarily to the CNT in the rabbit [117,118]. In other rodents, expression of TRPV5 is found in the DCT2 segment [expressing NCC (Na^+–Cl^- co-transporter) and ENaC (epithelial Na^+ channel)], the CNT and the initial CD [118–121]. The channel co-localizes with the Ca^{2+}-binding protein calbindin-D_{28K} and the basolateral extrusion proteins PMCA1b (plasma membrane ATPase 1b) and NCX1 (Na^{2+}/Ca^{2+} exchanger type 1). Transgenic mice lacking TRPV5 have augmented renal Ca^{2+} loss, and micropuncture studies effectively demonstrate that Ca^{2+} reabsorption in the distal convolutions is disturbed [122]. Several studies have shown TRPV5 as an essential constituent in transcellular Ca^{2+} reabsorption and it is, therefore, surprising that no monogenic disorders or polymorphisms have been identified thus far [123].

Detailed physiological measurements suggest that the distal convolutions are responsible for reabsorbing 5–6 % of the filtered Mg^{2+} [19,124]. Again, these values were determined by subtracting the delivery of ions to the distal sites and the final urine. Similar experiments have reported that approx. 8 % of the filtered load is reabsorbed between the early and late distal collections [115]. Brunette et al. [124] suggested that, on the basis of their early and late distal micropuncture results, reabsorption of Mg^{2+} is localized in the early portion rather than along the whole distal tubule, thus, predominantly in the DCT. The early distal tubule constitutes a high-resistance epithelium with a lumen negative voltage of approx. −5 mV [55,110,111]. Moreover, the transepithelial movement of Mg^{2+} appears to be unidirectional. These observations support the presence of active transcellular Mg^{2+} transport in this segment [125]. This is corroborated further by the observation that the luminal Mg^{2+} concentration in the DCT ranges between 0.2 and 0.7 mmol/l [4], while that of the cell is maintained approx. 0.2–1.0 mmol/l [126,127]. Under these conditions, uptake of Mg^{2+} across the apical membrane would be dictated largely by the negative intracellular membrane potential found in the DCT cell [128,129]. In addition, distal reabsorption of Mg^{2+} appears to be load-dependent, as increasing delivery augments reabsorption [54,125]. Cellular influx of Mg^{2+} probably occurs through the TRPM6 (TRP melastin 6) channel, a channel selective for divalent cations [130]. TRPM6 is structurally similar to other TRP channels with six transmembrane segments. Only shared with TRPM7 is the unique α-kinase domain fused to the C-termini [131]. It is worth noting that the current debate questions whether TRPM6 functions as a homo-tetramer. Distinctive properties of functional homomeric TRPM6 channels, as well as TRPM6/TRPM7 heteromeric channels, have been recorded [132]. In addition, several groups have reported the electrophysiological characteristics of TRPM6 in mammalian cells without simultaneously expressing TRPM7 [130,132,133]. In contrast with these observations, others have reported that TRPM7 expression is necessary for localization at the plasma membrane and for the generation of TRPM6 currents [134–137]. Thus the exact molecular composition of the Mg^{2+} influx pathway in transporting epithelia remains to be determined. *Trpm6* mRNA expression was restricted to the kidney, lung and intestine (with the highest abundance in colon and caecum of mice) [138,139]. Immunohistochemical localization of the channel has been reported, where TRPM6 was restricted to the apical membrane domains of renal DCT cells [130]. Intracellular carrier proteins and basolateral excursion mechanisms are yet to be identified.

TRANSPORT DEFECTS OF THE DCT AFFECTING DIVALENT CATION HANDLING

HSH (hypomagnesaemia with secondary hypocalcaemia)

TRPM6 was originally identified as the causative gene of the autosomal-recessive disorder known as HSH [139,140]. Individuals suffering from HSH present in early infancy with symptoms related to neuromuscular excitability, such as muscle spasms, tetany and generalized convulsions [141]. These symptoms probably occur as a consequence of a severe fall in plasma Mg^{2+} and Ca^{2+} concentrations. Symptoms can be reduced

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by high dose Mg^{2+} administration. Interestingly, this Mg^{2+} supplementation normalizes the plasma Ca^{2+} concentration, while plasma Mg^{2+} remains subnormal [142]. A patient with HSH had a failure to effectively absorb Mg^{2+} from low intraluminal intestinal Mg^{2+} concentrations [143]; however, the patient with HSH had a positive Ca^{2+} balance, excluding a common defect in Mg^{2+} and Ca^{2+} transport mechanisms. These results support a role for TRPM6 in saturable active Mg^{2+} transport, serving as an apical entry mechanism for Mg^{2+} in the enterocyte. Although the hypomagnesaemia in HSH appears to be primarily the result of malabsorption of Mg^{2+} in the intestine, there is also a serious, although not well studied, renal Mg^{2+} leak. Patients with HSH have been reported to have either normal renal Mg^{2+} conservation [142–144] or an additional renal Mg^{2+} leak [139,140,145]. However, when an intravenous Mg^{2+} load was administered to patients with HSH, renal wasting was clearly noticeable [140]. Thus these individuals waste urinary Mg^{2+} at a lower plasma concentration than normal individuals do. Agus [146] has described previously that maintaining a fractional Mg^{2+} excretion above 2 % in the presence of hypomagnesaemia should be considered a renal leak in itself. This is corroborated by a report showing an inappropriately high fractional excretion given severe hypomagnesaemia in patients with HSH [139].

IRH (isolated autosomal-recessive hypomagnesaemia)

This rare form of hypomagnesaemia was initially described in two siblings who presented with low serum Mg^{2+} concentrations and an inappropriately high fractional excretion of Mg^{2+} [147]. In addition, these individuals suffered from psychomotor retardation during their youth and are presently suffering from moderate mental retardation and epileptic seizures [147,148]. Thus these observations indicated a potential defect largely related to renal Mg^{2+} transport, similar to that observed for Mg^{2+}-loaded patients with HSH. Interestingly, there were no observable secondary changes in the handling of Ca^{2+} [147], which may to some extent explain why these patients are devoid of tetany. Detailed homozygosity mapping approaches and direct sequencing revealed that these sisters had a mutation in the *EGF* (epidermal growth factor) gene, causing a defect in the routing of the pro-EGF to the basolateral plasma membrane [148]. This mutation resulted in impaired autocrine/paracrine secretion of EGF to the interstitium, thereby inhibiting the activation of basolateral EGFRs (EGF receptors) in the DCT. Subsequent patch–clamp analysis and classical biochemical experiments showed that application of EGF activates TRPM6 and shuttles it from endomembrane compartments to the membrane [148,149]. Interestingly, inhibition of the EGFR by cetuximab, a monoclonal anticancer drug, causes pronounced hypomagnesaemia in colorectal cancer patients [148,150]. Unlike patients with impaired sorting of the EGF protein, cetuximab treatment produces secondary hypocalcaemia in patients with moderate-to-severe hypomagnesaemia (grade 2 or higher), probably resulting from impaired PTH secretion [150]. Similarly, patients with severe hypomagnesaemia did show signs of fatigue and cramps. IRH and related inhibition of the EGFR by cetuximab diverges from HSH in several of the phenotypic characteristics, suggesting perhaps additional effects of EGF on Mg^{2+} handling. Incubation of TRPM6-expressing cells with cetuximab completely abolishes the EGF-induced increases in channel currents [149]. Unlike TRPM6, the current literature suggests that TRPM7 is not activated by EGF and thus appears to be an unlikely target [149]. Whether other transporters are involved in conferring hypomagnesaemia in IRH remains to be defined. Taken together, these results indicate that EGF is a novel magnesiotropic factor acting primarily to increase renal Mg^{2+} reabsorption.

Isolated dominant hypomagnesaemia

This form of hypomagnesaemia was characterized in two unrelated families [151]. Affected individuals showed isolated renal Mg^{2+} wasting, as the renal reabsorption of Mg^{2+} remained within the normal range in the presence of hypomagnesaemia. The only other observable renal symptom was a lowered excretion of Ca^{2+} in the urine [151]. The underlying defect was later described to be a G41R mutation in the *FXYD2* gene, the so-called Na^+/K^+-ATPase γ-subunit. FXYD proteins are a family of single transmembrane proteins known to modulate Na^+/K^+-ATPase function. The exact intrarenal localization of the FXYD2 protein is controversial, although some reports suggest that it localizes to the DCT [152–155]. The FXYD2 protein changes the Na^+/K^+-ATPase kinetics by reducing the affinity for Na^+ while increasing that for K^+ [152,156,157]. FXYD2 G41R proteins have been shown to oligomerize, similar to the wild-type subunit. However, although FYXD2 localizes to the cytoplasm and the plasma membrane, the G41R mutant is retained in the cell. Thus it is suggested that the dominant-negative FXYD2 G41R protein would oligomerize with wild-type FXYD2 proteins, retaining them inside the cell unable to bind and activate the other Na^+/K^+-ATPase subunits [158,159]. Thus the current hypothesis states that reducing outward basolateral Na^+ transport in the polarized DCT cell reduces the intracellular voltage, thereby reducing the inward electric driving force for Mg^{2+} potentially through TRPM6. Interestingly, mice with a targeted disruption of the *Fxyd2* gene have no change in the urinary excretion of Mg^{2+} or serum Mg^{2+} concentrations [154]. The absence of hypomagnesaemia is also observed by heterozygous deletion of the entire *FXYD2* gene in humans, indicating

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that renal Mg^{2+} loss in this disease is due to a dominant-negative nature of the G41R mutation, rather than haploinsufficiency [158]. A recent report [160] suggests a potential role for FXYD2 as an inward rectifying channel, which differs from that of the FXYD2 G41R mutant. Moreover, the authors speculate that the FXYD2 protein can be involved in the basolateral extrusion of Mg^{2+} from the cell [160]. The exact role of the γ-subunit in regulating renal Mg^{2+} reabsorption remains to be clarified experimentally.

Gitelman's syndrome

Gitelman's syndrome is a salt-losing disorder characterized by hypokalemic metabolic alkalosis, hypomagnesaemia and hypocalciuria [161]. Renin activity and aldosterone concentrations are elevated, but only marginally in comparison with patients with Bartter's syndrome. The disorder is autosomal-recessive and is caused by mutations in *SLC12A3*, which encodes the thiazide-sensitive NCC [162]. The Gitelman's phenotype is mimicked by chronic thiazide treatment, a potent blocker of NCC [163,164]. The hypocalciuric effect of *SLC12A3* gene deletion or chronic thiazide administration is probably due to hypovolaemia, although some reports have suggested an additional direct effect on Ca^{2+} reabsorption in the distal convolutions [165,166]. Volume contraction causes secondary increases in proximal tubular Na^{+} transport and, thus, facilitates paracellular hyperabsorption of Ca^{2+}. This is supported by several observations. Micropuncture studies in mice with genetic ablation of the *Slc12a3* gene had a reduced delivery of Na^{+} and Ca^{2+} to the late proximal tubule, consistent with increased Na^{+} and Ca^{2+} reabsorption in the proximal tubule. In addition, these micropuncture experiments indicated that Ca^{2+} reabsorptive rates were similar in the distal convolutions in the wild-type and *Slc12a3*-deficient mice [167]. In wild-type mice administered thiazide diuretics, similar observations were made using micropuncture studies, suggesting that the hypocalciuric effect localizes to the proximal tubule [168]. Furthermore, thiazide-induced hypocalciuria can still be observed in *Trpv5*-deficient mice, which effectively lack Ca^{2+} transport in the distal convolutions [168]. This satisfactorily confirms that the mechanism by which hypocalciuria develops after thiazide usage and probably also in Gitelman's syndrome is independent of distal Ca^{2+} reabsorption.

Hypomagnesaemia is a consistent feature of Gitelman's syndrome. The phenotypic characteristic is likely to be the result of a decreased abundance of TRPM6, leading to renal Mg^{2+} wasting. However, the mechanism that decreases renal TRPM6 expression in Gitelman's syndrome remains incompletely understood. Mice deficient in NCC, as well as rats given thiazides, have severe atrophy of the DCT (i.e. the fractional volume of the early DCT is drastically reduced) [167,169]. In addition, *Slc12a3*-deficient mice have a decreased expression of TRPM6, which localizes intrarenally solely within the DCT [168]. Interestingly, in contrast with previous results, administration of lower doses of thiazides produce no changes in the morphology of the DCT, while the expression of TRPM6 remains decreased [168]. Aldosteronism, a recurring feature of Gitelman's syndrome, has been implicated in renal Mg^{2+} wasting [170–172]. Similarly, a lack of aldosterone can be associated with hypermagnesaemia [170,171]. Spironolactone, a mineralocorticoid receptor antagonist, has been shown to consistently reduce urinary Mg^{2+} excretion in a number of patient groups [170,171,173]. In patients with Gitelman's syndrome, spironolactone treatment increases serum Mg^{2+} concentrations and reduces the fractional tubular excretion of Mg^{2+} [173]. Thus some studies have suggested that the apparent aldosterone excess observed in Gitelman's syndrome may be an underlying cause. Conversely, chronic infusion of aldosterone does not change the renal mRNA abundance of *Trpm6* in mice [172]. Moreover, marked hyperaldosteronism is observed in patients with antenatal Bartter's syndrome, while less than 20 % of these individuals display hypomagnesaemia. The positive effect of spironolactone on Mg^{2+} balance deserves further research. Currently, the absence of NCC in DCT has been shown to induce hypocalciuria via changes in proximal tubular reabsorption and hypomagnesaemia by reducing TRPM6 expression.

TRANSPORT DEFECTS OF THE DISTAL CONVOLUTIONS AFFECTING DIVALENT CATION HANDLING

dRTA (distal renal tubular acidosis)

Metabolic acidosis caused by an inability of the distal tubule to effectively secrete H^{+} ions is referred to as dRTA. dRTA and other types of metabolic acidosis are often accompanied by hypokalaemia, hypercalciuria, nephrocalcinosis and metabolic bone disease. dRTA can be hereditary in an autosomal-dominant fashion or occur as a rarer recessive form which may be associated with sensorineural deafness. The underlying gene defect of dominant dRTA is due to mutations in *SLC4A1* [encoding band 3 erythrocyte AE1 (anion exchanger 1)] [174], whereas mutations in *ATP6V1B1* and *ATP6V0A4* (encoding subunits of the proton pump H^{+}-ATPase) cause recessive dRTA with [175] or without [176] sensorineural deafness respectively. It has long been known that metabolic acidosis affects Ca^{2+} handling. Persistent acidosis is buffered by alkaline salts released from bone, thereby promoting Ca^{2+} release from the hydroxyapatite crystal. These effects ultimately lead to osteomalacia and osteoporosis and may promote hypercalciuria [177]. Micropuncture experiments, show that distal tubular Ca^{2+} reabsorption is inhibited by

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chronic metabolic acidosis [178], suggesting that renal Ca^{2+} wasting is not merely due to Ca^{2+} mobilization from bone, but also constitutes an additional renal component. These findings are in good agreement with the observation that the renal expression of TRPV5 is decreased during experimental metabolic acidosis. In line with this, renal Ca^{2+} excretion is refractory to NH_4Cl-induced metabolic acidosis in *Trpv5*-deficient mice [179]. Thus the effect of impaired distal secretion of protons and other disturbances (such as renal failure, liver cirrhosis etc.) that cause metabolic acidosis affects the renal tubular transport of Ca^{2+}. Interestingly, Ca^{2+} influx, probably as the result of acute changes in subcellular distribution and of single channel properties of TRPV5, is regulated by the luminal pH, i.e. acidification of the medium inhibits TRPV5-mediated Ca^{2+} reabsorption [180,181]. Thus, during conditions of increased distal tubular proton secretion or delivery (metabolic acidosis due to causes other than dRTA), these TRPV5-specific effects probably also contribute to the observed hypercalciuria.

During dRTA, failure to effectively lower the intraluminal pH in the CNT/CD causes metabolic acidosis. During regular metabolic acidosis, urinary pH becomes more acidic to compensate for the decreased systemic pH; however, hypercalciuria is observed in both conditions. As experimental metabolic acidosis involves a renal component, this probably also occurs in dRTA. This is supported by the observation that most patients with an AE1 defect develop nephrocalcinosis, while few show signs of bone disease (although minor losses from bone could promote Ca^{2+} wasting) [174]. Furthermore, acetazolamide induces metabolic acidosis by reducing proximal tubule HCO_3^- reabsorption and, hence, increasing urinary pH, a situation similar to dRTA [182]. However, as observed with NH_4Cl treatment, acetazolamide administration causes the down-regulation of TRPV5 in mice, and the hypercalciuria associated with acetazolamide-induced acidosis is absent in *Trpv*5-deficient mice. Hence changes in systemic pH probably promote the down-regulation of renal TRPV5 expression. In contrast, chronic metabolic alkalosis is associated with increased Ca^{2+} reabsorption and TRPV5 abundance. However, the Ca^{2+}-sparing effect is also observed in *Trpv5*-deficient mice, suggesting that another unknown mechanism is responsible for the increased Ca^{2+} reabsorption [179].

In patients with dRTA, serum Mg^{2+} concentrations remains unaffected [174,183]; however, children with dRTA present with hypermagnesuria, which can be corrected by HCO_3^- administration [183]. Detailed clearance and micropuncture studies have shown that chronic metabolic acidosis causes hypermagnesuria beyond the proximal tubule [184]. Furthermore, chronic metabolic acidosis decreases the renal abundance of TRPM6, whereas metabolic alkalosis has the opposite effect [179]. Acidic pH also augments the inward current of TRPM6, suggesting that perhaps acute changes in intraluminal pH may affect the channel in an opposite manner from chronic metabolic acidosis. The physiology of this observation is still incompletely understood [132].

PHAII (pseudohypoaldosteronism type II)

The WNK (with-no-lysine) kinase family consists of serine/threonine kinases with a characteristic displacement of a catalytic lysine residue necessary for ATP binding [185,186]. The identification of the WNK family members as multifunctional proteins required for ambient BP maintenance and renal electrolyte handling has progressively broadened the knowledge of how these processes are regulated [187,188]. PHAII, also known as Gordon's syndrome, is an autosomal-dominant disorder associated with hypertension, an augmented renal reabsorption of Na^+/Cl^- and impaired secretion of K^+ and H^+ [188,189]. The genetic defects in PHAII are due to loss-of-function mutations in *WNK4*, as well as activating mutations in *WNK1* caused by intronic deletions [188]. Several studies have now shown that WNK1 and WNK4 play important roles in modulating electrolyte transport pathways in the distal convolutions [190–193].

In addition to the often hypertensive phenotype, hypercalciuria and nephrolithiasis has been reported in patients with PHAII [194,195]. Accordingly, individuals carrying a *WNK4* Q565E mutation present with hypercalciuria and a decreased bone mineral density. Conversely, affected members with activating *WNK1* mutations present with normocalciuria [196]. It is interesting to note that no apparent change is observed in serum Mg^{2+} concentrations or renal Mg^{2+} handling in patients with PHAII [196–198]. The mechanism by which dysfunctional WNK4 stimulates calciuria in PHAII is unclear, but may occur via several mechanisms. (i) Secondary to volume expansion, a condition that is predicted to reduce proximal tubule Na^+ reabsorption, resulting in decreased electrochemical driving force for Ca^{2+} uptake across the proximal tubular epithelia [16,168,199]. As the majority of Ca^{2+} reabsorption occurs in the proximal tubule, volume expansion might be the largest contributor to hypercalciuria in PHAII. Even so, individuals carrying the WNK1 deletion have normocalciuria in the presence of elevated BP (and probably an expanded extracellular volume). (ii) Alternatively, the hypercalciuric phenotype in PHAII may be due to impaired interactions between WNK4 and renal Ca^{2+} transport proteins [200]. In line with this, it has been reported that WNK4 directly activates Ca^{2+} transport by increasing the surface expression of TRPV5 in the *Xenopus* oocyte expression system [200]. However, the *WNK4* PHAII mutants showed a similar activating effect on TRPV5. Using lentivirus-induced gene delivery to primary rabbit CNT/cortical CD cells, we found that cells infected with WNK4 and the WNK4 Q565E

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mutant both decreased transepithelial Ca^{2+} transport (H. Dimke, J.G. Hoenderop and R.J. Bindels, unpublished work). Consequently, the exact aetiology underlying hypercalcaemia in PHAII needs further clarification. It is interesting to note that, although PHAII is considered a mirror image of Gitelman's syndrome, no changes in serum Mg^{2+} have been observed in these individuals.

COLLECTING SYSTEM

It is generally accepted that the CD does not contribute significantly to overall renal Ca^{2+} transport. Classical micropuncture studies have been aided by determining the fraction of Ca^{2+} transport after the distal convolutions, by allowing late distal measurements of cations. On the basis of the observations that (i) relatively little K^+ secretion is thought to occur in the early distal convolutions comprising the DCT and (ii) K^+ secretion coupled with increased osmotic water permeability in CNT and accessible CD segments markedly raises luminal K^+ concentration, it was possible to correlate the fractional delivery of Ca^{2+} to the luminal K^+ concentration at distal puncture sites. As evident from the intrarenal distribution of TRPV5, Ca^{2+} delivery decreases with increased luminal K^+ concentration in wild-type mice. Conversely, in mice with a targeted disruption of the *Trpv5* gene, Ca^{2+} delivery was markedly increased with increasing luminal K^+ concentrations [122]. Results obtained from these experiments and others suggest that very little Ca^{2+} ($<2\%$) is delivered to segments of high K^+ secretion [122,168]. Assuming that the highest luminal K^+ concentration represents the very latest superficial distal convolutions, negligible Ca^{2+} transport must occur in the remainder of the CD. Microperfusion of the granular and light portions of the cortical ducts in the rabbit have suggested that Ca^{2+} transport only occurs in the granular portion. Thus the non-superficial segments of the collecting system probably provide a small contribution to overall renal Ca^{2+} reabsorption.

The relative contributions of the CD to overall Mg^{2+} reabsorption are probably negligible. Estimations from micropuncture studies suggest little Mg^{2+} transport after the DCT. Microperfusion of cortical CDs failed to show a significant amount of net Mg^{2+} movement [201]. In line with this, microcatheterization studies found no net Mg^{2+} transport over the length of the inner medulla CD [202].

HYPOMAGNESAEMIA, HYPERTENSION AND HYPERCHOLESTEROLAEMIA

A large family was identified with a high prevalence of hypomagnesaemia, hypertension, and hypercholesterolaemia [203]. Interestingly, males with the syndrome did not transmit the phenotype to their offspring. Conversely, offspring from affected females inherited the trait with high frequency, suggesting mitochondrial transmission. The defect was discovered to be a thymidine-to-cytidine conversion near the anticodon of the isoleucine tRNA. Subjects suffering from hypomagnesaemia had an augmented fractional excretion of Mg^{2+}, indicative of renal wasting. Individuals in the maternal lineage also had renal Ca^{2+} loss. Hypokalaemia was present in subjects with hypomagnesaemia, perhaps as a consequence of hypomagnesaemia. In one affected member, immunohistochemical and electron microscopic evaluation revealed signs of mitochondrial dysfunction. In line with this, NMR revealed a decreased production of ATP in skeletal muscle [203].

The exact aetiology underlying this disorder remains unclear. The authors suggested that reduced energy production could limit the highly energy consuming DCT and thereby impede transcellular Mg^{2+} transport [109]. How large a reduction would be needed to diminish the membrane potential enough to affect Mg^{2+} reabsorption in the DCT is unclear; however, the renal Na^+ excretion remained unchanged between the affected and unaffected family members [203].

SUMMARY

The necessity of maintaining the systemic concentrations of Ca^{2+} and Mg^{2+} within normal limits is evident, as disturbances in these divalent cations affect important physiological processes. For instance, hypomagnesaemia is a recurrent condition often encountered in the clinical setting. The detailed characterization of the underlying causes of monogenic diseases related to defective renal Ca^{2+} and Mg^{2+} handling has greatly aided the understanding of how renal and, hence, systemic divalent homoeostasis is maintained. Proximal tubular transport defects, such as Dent's disease, can cause hypercalciuria. In the TAL, inadequate, absent or even increased ion transport confers several syndromes, which drastically changes the renal handling of Ca^{2+} and Mg^{2+}. In addition, vectorial transcellular Ca^{2+} and Mg^{2+} transport in the distal convolutions can be disturbed as a consequence of changes in the transport of other ions or by directly affecting TRP channel function. An even better understanding of the mechanisms governing the transport of Ca^{2+} and Mg^{2+} can be obtained by studying these disease conditions or by mimicking these in genetically modified animals.

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