Activation of the Ca$^{2+}$-sensing receptor increases renal claudin-14 expression and urinary Ca$^{2+}$ excretion

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Dimke H, Desai P, Borovac J, Lau A, Pan W, Alexander RT. Activation of the Ca$^{2+}$-sensing receptor increases renal claudin-14 expression and urinary Ca$^{2+}$ excretion. Am J Physiol Renal Physiol 304: F761–F769, 2013. First published January 2, 2013; doi:10.1152/ajprenal.00263.2012.—Kidney stones are a prevalent clinical condition imposing a large economic burden on the healthcare system. Hypercalciuria remains the major risk factor for development of a Ca$^{2+}$-containing stone. The kidney’s ability to alter Ca$^{2+}$ excretion in response to changes in serum Ca$^{2+}$ is in part mediated by the Ca$^{2+}$-sensing receptor (CaSR). Recent studies revealed renal claudin-14 (Cldn14) expression localized to the thick ascending limb (TAL) and its expression to be regulated via the CaSR. We find that Cldn14 expression is increased by high dietary Ca$^{2+}$ intake and by elevated serum Ca$^{2+}$ levels induced by prolonged 1,25-dihydroxyvitamin D$_{3}$ administration. Consistent with this, activation of the CaSR in vivo via administration of the calcimimetic cinacalcet hydrochloride led to a 40-fold increase in Cldn14 mRNA. Moreover, overexpression of Cldn14 in two separate cell culture models decreased paracellular Ca$^{2+}$ flux by preferentially decreasing cation permeability, thereby increasing transepithelial resistance. These data support the existence of a mechanism whereby activation of the CaSR in the TAL increases Cldn14 expression, which in turn blocks the paracellular reabsorption of Ca$^{2+}$. This molecular mechanism likely facilitates renal Ca$^{2+}$ losses in response to elevated serum Ca$^{2+}$. Moreover, dysregulation of the newly described CaSR-Cldn14 axis likely contributes to the development of hypercalciuria and kidney stones.

Hypercalciuria; CaSR; kidney stones; nephrolithiasis; thick ascending limb

The prevalence of kidney stones is increasing (33). In some populations, kidney stones have been observed in as many as 12% of men and 5% of women (5). The occurrence of symptomatic stones is strongly dependent on age and race (33), and the recurrence rate is high. Stone disease is expensive to treat due to frequent emergency room visits, hospitalizations, and surgeries. Treatment costs 5 billion dollars annually in the United States alone (30). Moreover, stone disease is directly associated with an increase in the likelihood of adverse renal outcomes, including end-stage renal disease (1).

The greatest risk factor for the development of a Ca$^{2+}$-containing stone is hypercalciuria (22). Notably, ~80% of stones are calcium (Ca$^{2+}$) containing (5). Increased urinary Ca$^{2+}$ excretion contributes to Ca$^{2+}$ crystal growth (5). Conversely, a reduction in urinary Ca$^{2+}$ excretion slows stone formation (10, 21). Hypercalciuria may result from disturbed transport of Ca$^{2+}$ in several organs. Increased intestinal ab-

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disregulation provides a likely molecular explanation as to why SNPs in the CLDN14 gene correlate with kidney stones and lower bone mineral density (34).

MATERIALS AND METHODS

Experimental protocol 1. FVB/N mice (Jackson Laboratories, Bar Harbor, ME) were fed a low (0.01%, TD.95027), normal (0.6%, TD.97191), or high (2%, TD.00374)-Ca\(^{2+}\) diet for either 10 or 21 days (n = 48, 8/group). Diets were custom made by Harlan Laboratories (Madison, WI). Mice were housed in metabolic cages at the end of the experimental period for a 24-h urine collection. On the last day, animals were anesthetized using pentobarbital sodium, and blood was collected by cardiac puncture (Sensipar, Amgen) in food at a dose of 1 mg/g body weight. Animals were maintained on a standard diet with ad libitum access to food and water. Animals were kept in regular cages or vehicle. The mice were maintained on a standard diet with ad libitum access to food and water. Animals were kept in regular cages or vehicle. The mice were maintained on a standard diet with ad libitum access to food and water. Animals were kept in regular cages or vehicle. The mice were maintained on a standard diet with ad libitum access to food and water. Animals were kept in regular cages or vehicle. The mice were maintained on a standard diet with ad libitum access to food and water. Animals were kept in regular cages or vehicle. The mice were maintained on a standard diet with ad libitum access to food and water. Animals were kept in regular cages or vehicle. The mice were maintained on a standard diet with ad libitum access to food and water. Animals were kept in regular cages or vehicle. The mice were maintained on a standard diet with ad libitum access to food and water. Animals were kept in regular cages or vehicle. The mice were maintained on a standard diet with ad libitum access to food and water. Animals were kept in regular cages or vehicle. The mice were maintained on a standard diet with ad libitum access to food and water. Animals were kept in regular cages or vehicle. The mice were maintained on a standard diet with ad libitum access to food and water. Animals were kept in regular cages or vehicle. The mice were maintained on a standard diet with ad libitum access to food and water. Animals were kept in regular cages or vehicle. The mice were maintained on a standard diet with ad libitum access to food and water. Animals were kept in regular cages or vehicle. The mice were maintained on a standard diet with ad libitum access to food and water. Animals were kept in regular cages or vehicle. The mice were maintained on a standard diet with ad libitum access to food and water. Animals were kept in regular cages or vehicle. The mice were maintained on a standard diet with ad libitum access to food and water. Animals were kept in regular cages or vehicle. The mice were maintained on a standard diet with ad libitum access to food and water. Animals were kept in regular cages or vehicle.

Experimental protocol 2. FVB/N mice (n = 8/group) were placed on a standard diet with ad libitum access to food and water. During the last 48 h of the experimental period, mice were placed in metabolic cages. Mice were maintained and processed exactly as described in experimental protocol 1.

Experimental protocol 3. FVB/N mice (n = 6/group) were given capecitabine (Sensipar, Amgen) in food at a dose of 1 mg/g body weight or vehicle. The mice were maintained on a standard diet with ad libitum access to food and water. Animals were kept in regular cages for 2 days and subsequently placed in metabolic cages for the remaining 4 days of the study. The mice were then processed as described in experimental protocol 1.

Determination of solutes, creatinine, and hormones. Urinary Ca\(^{2+}\) was determined using a colorimetric assay kit (Quantichrom TM Ca\(^{2+}\) Assay Kit, BioAssay System, Hayward, CA). Urinary creatinine was measured using a Creatinine Parameter Assay Kit (R&D Systems, Minneapolis, MN). Intact plasma parathyroid hormone (PTH) levels were determined with a mouse PTH ELISA kit (Immunotopes International, San Clemente, CA), and serum 1,25(OH)\(_2\)D\(_3\) concentrations were determined by a radioimmunoassay (RIA) kit (Immunodiagnostic Systems, Fountain Hills, AZ).

Real-time PCR. Total RNA was isolated from kidneys or cells using TRIzol Reagent (Invitrogen, Carlsbad, CA) reverse transcribed into cDNA, which was used to determine gene expression as described in detail previously (25). Primers and probes were made by Integrated DNA Technologies (San Diego, CA). Sequences for primers used to evaluate expression of Cldn14 were as follows: mCldn14: forward primer 5'-TGGCATGAGGTGTTAATCCG-3'; probe 5'-TGAGGAGACAGGGATGAGGAGATGAAGC-3'; reverse primer CG-GTGGATGATGGTTAGGG; expression levels were quantified with the ABI Prism 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA).

Cell culture studies. Opossum kidney (OK) cells were grown and maintained as described previously (25). Monoclonal stable cell lines expressing mouse Cldn14 under control of the Tet-off system were generated after the cells were grown to confluence (5–7 days) and then fixed using 4% paraformaldehyde (PFA). After being quenched with 5% glycine, the cells were permeabilized with 0.2% Triton X-100. Primary anti-Myc antibody (9B11, Cell Signaling Technology) and rabbit anti-zonula occludens (ZO-1; Invitrogen) were first applied. After washing, secondary DiLight 549 AffiniPure donkey anti-mouse and DiLight 488 AffiniPure donkey anti-rabbit conjugated antibodies (both from Jackson ImmunoResearch Laboratories) were applied at a dilution of 1:500 for 1 h at room temperature. Specimens were analyzed using a spinning disc confocal microscope (WaveFx, Quorum Technologies, Guelph, Canada).

Ussing chambers. Myc-tagged Cldn14-expressing cells and mock-transfected cells were seeded onto Snapwell inserts (Corning, NY) and grown to confluence. Ussing chamber studies were carried out as described previously (4). Initially, we corrected for the baseline conditions of empty Ussing chambers with buffer A (145 mM NaCl, 2 mM CaCl\(_2\), 1 mM MgCl\(_2\), 10 mM glucose, and 10 mM HEPES, pH 7.4). The Snapwell inserts with confluent OK monolayers were washed three times using buffer A and then mounted between the two hemichambers, both of which were filled with 10 ml of buffer A. Current clamps were performed using a DVC 1000 IV Clamp (World Precision Instruments, Sarasota, FL), and electrodes containing an agarose bridge with 3 M KCl. Data were acquired as a trace and recorded using PowerLab (ADInstruments, Colorado Springs CO) running Chart 4.0 software. To determine the transepithelial resistance (TER) and permeability properties of the epithelia, a 90-μA current was applied across each monolayer and a dilution potential was induced by replacing buffer A in the apical hemichamber with buffer B (80 mM NaCl, 130 mM mannitol, 2 mM CaCl\(_2\), 1 mM MgCl\(_2\), 10 mM glucose, and 10 mM HEPES, pH 7.4). The dilution potential and voltage of each filter were determined following removal of the cells by trypsinization (30 min, at 37°C), and this measurement was subtracted from the values generated by the filter containing cells. Goldman-Hodgkin-Katz and Kimizuka-Koketsu (18) equations were used as described previously (16) to calculate the absolute permeability of Na\(^+\) and Cl\(^-\) and to determine the relative permeability of Na\(^+\)-to-Cl\(^-\) (P\(_{NaCl}\)/P\(_{ClNa}\)) ratio.

Ca\(^{2+}\) flux studies. 45Ca\(^{2+}\) tracer (PerkinElmer) flux was determined in Ussing chambers with the transepithelial potential difference clamped to 0 mV and equimolar Ca\(^{2+}\) in each half of the hemichamber to prevent generating an electrochemical gradient for Ca\(^{2+}\) flux across the monolayer. Empty vector (mock)-transfected and cells expressing Myc-tagged Cldn14 were seeded onto Transwell permeable supports (Corning, Corning, NY) and grown for 7 days. 45CaCl\(_2\) (25 μCi/ml) was applied to the apical hemichamber, and samples were
**RESULTS**

*High dietary Ca\(^{2+}\) intake increases Cldn14 expression.* FVB/N mice were placed on a low (0.01%), normal (0.6%), or high (2%) Ca\(^{2+}\) diet for 10 days. Another group of mice was placed on the same diets for 21 days. The concentration of ionized Ca\(^{2+}\) in the blood was not different between groups (Fig. 1A). The urinary Ca\(^{2+}\)/creatinine (Ca\(^{2+}\)/Crea) ratio increased proportionally to the amount of Ca\(^{2+}\) in the diet (Fig. 1B). There was a significant reduction in serum PTH in mice on high dietary Ca\(^{2+}\) for 10 days (Fig. 1C). Mice maintained on a low-Ca\(^{2+}\) diet had significantly higher serum 1,25(OH)\(_2\)D\(_3\) levels (Fig. 1D). Consistent with this, renal expression of 1α-hydroxylase (1α-OHase), the enzyme responsible for the generation of active 1,25(OH)\(_2\)D\(_3\), significantly increased in mice maintained on a low-Ca\(^{2+}\) diet (Fig. 1E). Conversely, expression of vitamin D-24-hydroxylase (24-OHase), the enzyme that catabolizes active 1,25(OH)\(_2\)D\(_3\) into its inactive form, was significantly increased in animals maintained on a high-Ca\(^{2+}\) diet (Fig. 1F). Mice maintained on a high-Ca\(^{2+}\) diet had a 2.5-fold increase in renal expression of Cldn14 after 10 days and a similar increase after 21 days. We found no difference in renal Cldn14 expression in mice maintained on a low-Ca\(^{2+}\) diet vs. a normal diet for either period of time (Fig. 1G).

*Active 1,25(OH)\(_2\)D\(_3\) increases Cldn14 expression.* To determine the potential role of 1,25(OH)\(_2\)D\(_3\) on the expression of Cldn14, animals received daily injections of 1,25(OH)\(_2\)D\(_3\) or vehicle for 5 days. 1,25(OH)\(_2\)D\(_3\) promotes intestinal hyperab-

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**Fig. 1.** High dietary Ca\(^{2+}\) increases claudin (Cldn) 14 expression. A and B: serum ionized Ca\(^{2+}\) (A) and urinary Ca\(^{2+}\)/creatinine (Crea) ratio (B) in mice receiving a diet containing low (0.01%), normal (0.6%), or high (2%) Ca\(^{2+}\) content for either 10 or 21 days. Also shown are parathyroid hormone (PTH; C) and 1,25-dihydroxyvitamin D\(_3\) (1,25(OH)\(_2\)D\(_3\); D) levels measured in serum, renal mRNA expression of 1α-hydroxylase (1α-OHase; E) and D-24-hydroxylase (24-OHase; F) in mice on different dietary amounts of Ca\(^{2+}\), and renal Cldn14 mRNA expression (G). Values are means ± SE. *P < 0.05 relative to animals maintained on the control diet. #P < 0.05 relative to animals maintained on low dietary Ca\(^{2+}\).
sorption and renal transport of Ca\textsuperscript{2+}. Animals injected with 1,25(OH)_2D_3 developed significantly increased levels of ionized Ca\textsuperscript{2+} in the blood (Fig. 2A). The urinary Ca\textsuperscript{2+}/Crea ratio was also elevated, but did not reach statistical significance (Fig. 2B). Serum PTH was undetectable in the mice administered 1,25(OH)_2D_3, and 1,25(OH)_2D_3 levels were increased (Fig. 2, C and D). The renal expression of 1\alpha-OHase significantly decreased and 24-OHase was elevated (Fig. 2, E and F). Renal Cldn14 mRNA expression increased ~10-fold in animals injected with 1,25(OH)_2D_3 (Fig. 2G).

**Cldn14 expression is stimulated by calcimimetics in vivo.** Renal Cldn14 expression was elevated by a high-Ca\textsuperscript{2+} diet, a condition suppressing PTH and 1,25(OH)_2D_3. In contrast, administration of 1,25(OH)_2D_3 increased Cldn14 expression 10-fold while a low-Ca\textsuperscript{2+} diet had no affect on Cldn14 expression, even though it increased 1,25(OH)_2D_3 levels. Taken together, the data suggest that elevated serum Ca\textsuperscript{2+} is responsible for stimulating renal Cldn14 expression. To test this hypothesis, mice were administered the calcimimetic cinacalcet hydrochloride (cinacalcet). In these animals, ionized free Ca\textsuperscript{2+} in the blood was significantly reduced, as expected from CaSR hyperactivation (Fig. 3A). Consistent with this, animals receiving cinacalcet developed tetany by the end of the experimental period. The urinary Ca\textsuperscript{2+}/Crea ratio was significantly elevated in animals treated with cinacalcet (Fig. 3B). PTH levels were undetectable, and 1,25-(OH)_2D_3 levels were unaltered (Fig. 3, C and D). Renal expression of 1\alpha-OHase was decreased, and the expression of 24-OHase was increased (Fig. 3, E and F). Renal mRNA expression of Cldn14 was increased 40-fold in animals administered cinacalcet (Fig. 3G), consistent with the hypothesis that CaSR activation increases renal Cldn14 expression.

**Cldn14 forms a preferential cation barrier.** We hypothesized that overexpressing Cldn14 would increase TER. As such, we chose to overexpress it in a renal epithelial cell culture model with a very low TER, such as OK cells (4). OK cells were therefore stably transfectected with Cldn14 bearing a C-terminal Myc-tag. Dilution potential measurements were performed in confluent monolayers of cells expressing Myc-tagged Cldn14 or cells expressing the empty vector alone (Mock). Consistent with Cldn14 forming a preferential cation barrier, overexpression markedly reduced Na\textsuperscript{+} permeability, while the permeability to Cl\textsuperscript{−} did not decrease significantly (Fig. 4, A and B). Together, this resulted in a decreased P_Na/P_Cl ratio (Fig. 4C), and a pronounced decrease in the transepithelial flux of Ca\textsuperscript{2+} (Fig. 4D). In line with this, overexpression of

![Figure 2](http://ajprenal.physiology.org/)

**Fig. 2.** 1,25(OH)_2D_3 administration increases renal Cldn14 abundance. A and B: serum ionized Ca\textsuperscript{2+} (A) and urinary Ca\textsuperscript{2+}/Crea ratio (B) in mice injected ip with 1,25(OH)_2D_3 for 5 days. Also shown are serum levels of PTH (C) and 24-OHase (D), renal mRNA expression of 1\alpha-OHase (E) and 24-OHase (F) in animals treated with 1,25(OH)_2D_3 or vehicle (Con), and expression of renal Cldn14 mRNA in response to chronic administration of 1,25(OH)_2D_3 (G). Values are means ± SE. PTH was not detectable (ND) in mice administered 1,25(OH)_2D_3. *P < 0.05 relative to animals receiving vehicle injection (control).
Cldn14 caused a clear increase in TER (Fig. 4E). Immunoblotting of whole cell lysate for Myc detected a single band of ~25 kDa, the predicted size of Cldn14, which was absent in mock-transfected cells (Fig. 4F). Expression of Cldn14 mRNA was dramatically increased in the OK cells transfected with the Myc-tagged Cldn14 construct (Fig. 4G). Immunofluorescence staining with α-Myc revealed expression of Cldn14 at cell-cell contacts, where it colocalized with ZO-1 (Fig. 4H), consistent with localization to the tight junction. In an effort to determine whether Cldn14 expression affected the expression of other claudins, we evaluated the mRNA abundance of claudins known to be expressed in the OK cell line (4). We found that overexpression of Cldn14 did not alter the expression of claudins -1, -4, -9, -12, -15, or -20. However, it induced a threefold increase in Cldn6 expression (Fig. 4I).

To verify the functional role of Cldn14 in the tight junction, we also investigated the role of Cldn14 in MDCK II cells using the Tet-off system. The results are listed in Table 1. Overexpression of Cldn14 in this system yielded changes in the P_{Na}/P_{Cl} ratio, P_{Ca}, and TER comparable to those observed in OK cells, suggesting that preferential blockage of cations is a direct effect of increased Cldn14 expression. This is in line with data obtained previously by Ben-Yosef et al. (3).

**DISCUSSION**

Renal regulation of Ca^{2+} excretion is central to maintaining the serum Ca^{2+} concentration within a tight range. Alterations of this process can cause hypercalciuria, leading to the formation of Ca^{2+}-containing kidney stones (22). The CaSR, which is expressed in the basolateral membrane of the TAL, plays a central role in this process. However, the downstream mechanisms after CaSR activation remain incompletely elucidated. Here, we report that CaSR stimulation prevents paracellular Ca^{2+} flux by increasing renal Cldn14 expression. This is based on the following four observations.

1) Renal Cldn14 expression is increased with a high-Ca^{2+} diet, while unaltered when dietary Ca^{2+} is low or normal, suggesting that elevated free Ca^{2+} could be the main stimulator of Cldn14 expression.

2) Increased systemic concentrations of ionized Ca^{2+} observed after chronic 1,25(OH)_{2}D_{3} administration also leads to marked increases in Cldn14 expression.

3) Administration of the calcimimetic cinacalcet, which acts on the CaSR to increase its sensitivity to Ca^{2+}, potently induces a 40-fold increase in the abundance of Cldn14.

4) Overexpression of Cldn14 markedly increases transcellular resistance and decreases paracellular Ca^{2+} flux. Taken together, these data suggest a mechanism...
whereby increased circulating Ca\textsuperscript{2+} activates the CaSR, causing increased renal Cldn14 expression. This in turn blocks Ca\textsuperscript{2+}-permeable paracellular pores, preventing the increased amount of filtered Ca\textsuperscript{2+} from being reabsorbed back into the blood. Ultimately, a Ca\textsuperscript{2+} load is excreted, returning circulating Ca\textsuperscript{2+} to normal levels.

Multiple studies have described the renal localization of Cldn14 with conflicting results (3, 9, 19). Recently, a detailed

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**Fig. 4.** Cldn14 increases transepithelial resistance by blocking paracellular ion permeation. A and B: permeability measurements for Na\textsuperscript{+} and Cl\textsuperscript{−} across confluent monolayers of opossum kidney (OK) cells expressing Myc-tagged Cldn14 (black bars) or empty vector (Mock; white bars). C: Na-to-Cl permeability ratio (PNa/PCl) of OK cells stably expressing Myc-tagged Cldn14 or empty vector. D: \textsuperscript{45}Ca\textsuperscript{2+} flux across confluent monolayers of OK cells expressing Cldn14 or empty vector. E: transepithelial resistance (TER) of confluent monolayers of OK cells stably transfected with Myc-tagged Cldn14 or control. F: immunoblot of whole cell lysate from OK cells stably expressing Myc-tagged Cldn14 or mock-transfected cells. G: mRNA expression of mouse Cldn14 in OK cells stably transfected with mock or Cldn14. H: representative confocal images through XZ and XY planes of OK cells expressing Myc-tagged Cldn14, immunostained for zonula occludens (ZO)-1, Myc (Cldn14), and 4,6-diamidino-2-phenylindole (DAPI). Scale bars represent 8 μm in the XY planes and 1 μm in the Z-axis. I: mRNA abundance of claudins known to be endogenously expressed in OK cells in stable clones expressing either Myc-tagged Cldn14 or mock. Values are means ± SE. *P < 0.05 relative to control transfected cells; n ≥3 independent stable cell lines.
expression induced by high dietary Ca$^{2+}$.

Gong et al. (12) found that increased renal Cldn14 mRNA

neuvers markedly upregulated renal Cldn14 mRNA expres-

CaSR activation by different dietary or pharmacological ma-

transepithelial potential difference to values as high as 30 mV

of the cortical TAL, a process potentially further increasing the

that a similar relationship is present in our experiments.

creased renal Cldn14 protein abundance. We expect therefore

Na$^{+}$ influx into TAL epithelial cells. This contributes to a lumen-

to-blood Ca$^{2+}$ reabsorption from the TAL.

Estimates of the concentration of Ca$^{2+}$ at the bend of the

loop of Henle suggest it is almost double (~3 mM) that of

superficial proximal puncture sites (~1.8 mM) (2). A high

reabsorptive capacity for Ca$^{2+}$ from the TAL is evinced by

significantly lower concentrations of Ca$^{2+}$ in fluid obtained

from more distal puncture sites (2). Dietary Ca$^{2+}$ loading is

expected to increase the distal delivery of Ca$^{2+}$ by several-fold

(~3-fold in our dietary Ca$^{2+}$ experiments). During such

conditions, modification of the lumen-positive voltage would

likely not be enough to prevent significant paracellular reab-

sorption across the TAL due to the chemical gradient present

(lumen to blood). Consequently, altered paracellular P$_{c}$ likely

contributes significantly to decreased TAL Ca$^{2+}$ reabsorption

after activation of the CaSR.

SNPs in the CLDN14 gene correlate with kidney stone

formation, osteopenia, and hypercalciuria. How Cldn14 could

cause these abnormalities was not apparent, since a correlation

between the risk variants and mRNA expression of Cldn14 in

adipose and peripheral blood samples was not observed (34).

Very recent data (12) and this study together suggest that

significant renal Cldn14 expression is strongly dependent on

activation of the CaSR. Low baseline renal expression of

Cldn14 explains why patients lacking CLDN14 do not dem-

onstrate evidence of altered Ca$^{2+}$ homeostasis (i.e., stone

formation or osteopenia) (38), although detailed physiological

characterization may reveal that they are unable to effectively

excrete a Ca$^{2+}$ load. Moreover, the urinary excretion of Ca$^{2+}$

in Cldn11/Cldn14 double knockout mice was not altered,

providing further evidence that the absence of Cldn14 does not

impact renal Ca$^{2+}$ handling under normal dietary conditions

(9). A critical experiment confirming a role for Cldn14 in renal

Ca$^{2+}$ handling was recently performed (12). Cldn14-deficient

mice were placed on a high-Ca$^{2+}$ diet and found to have

relative hypomagnesuria and hypocalciuria compared with

wild-type controls (12). This response would be expected in the

absence of the CaSR-Cldn14 axis, as an increased lumen-
to-blood Ca$^{2+}$ concentration gradient would favor increased

paracellular Ca$^{2+}$ reabsorption from the TAL.

In some patients, autosomal dominant hypocalcemic hypercalciuria is a result of activating mutations in the CASR gene

(26, 27). Mild asymptomatic hypocalcemia is generally ob-

served in these patients (26). These mutations cause the half-

maximal activity of the receptor to increase (26, 27). More

severe activating mutations in the CaSR cause an autosomal

dominant form of Bartter’s syndrome (27, 35, 37). These

individuals have a classic Bartter-like phenotype but differ

clinically from individuals with classic Bartter as they have

decreased PTH, hypocalcemia, renal Ca$^{2+}$ wasting, and

nephrocalcinosis. Discovery of the CaSR-Cldn14 axis provides

further insight into these symptoms. Activating mutations in
the CaSR would increase Cldn14 expression inappropriately, causing renal Ca$^{2+}$ wasting and nephrocalcinosis. Polymorphisms in the CaSR have also been implicated in idiopathic hypercalciuria (36). As both Cldn14 and the CaSR are now part of a common pathway, association studies may increase their power by grouping CaSR and Cldn14 together. Finally, whether cinacalcet poses a potential risk for the development of kidney stones in patients with primary hyperparathyroidism remains to be established.

In conclusion, our results extend recently published data suggesting that Cldn14 is regulated via a novel CaSR-dependent mechanism (12). Based on these findings, it is likely that Cldn14 plays a key role in the regulation of renal Ca$^{2+}$ excretion. Elegant work from Gong et al. (12) suggests that micro-RNAs binding to the 3′-untranslated region of Cldn14 are regulated by CaSR signaling and in turn alter Cldn14 expression. Whether changes in the micro-RNA recognition sites are responsible for hypercalciuria in humans remains to be determined. Regardless, alterations in the CaSR-Cldn14 axis likely contribute importantly to the development of hypercalciuria and the formation of kidney stones.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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