Deletion of claudin-10 rescues claudin-16–deficient mice from hypomagnesemia and hypercalciuria

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The tight junction proteins claudin-10 and -16 are crucial for the paracellular reabsorption of cations along the thick ascending limb of Henle's loop in the kidney. In patients, mutations in *CLDN16* cause familial hypomagnesemia with hypercalciuria and nephrocalcinosis, while mutations in *CLDN10* impair kidney function. Mice lacking claudin-16 display magnesium and calcium wasting, whereas absence of claudin-10 results in hypermagnesemia and interstitial nephrocalcinosis. In order to study the functional interdependence of claudin-10 and -16 we generated double-deficient mice. These mice had normal serum magnesium and urinary excretion of magnesium and calcium and showed polyuria and sodium retention at the expense of increased renal potassium excretion, but no nephrocalcinosis. Isolated thick ascending limb tubules of double mutants displayed a complete loss of paracellular cation selectivity and functionality. Mice lacking both claudin-10 and -16 in the thick ascending limb recruited downstream compensatory mechanisms and showed hypertrophic distal convoluted tubules with changes in gene expression and phosphorylation of ion transporters in this segment, presumably triggered by the mild decrease in serum potassium. Thus, severe individual phenotypes in claudin-10 and claudin-16 knockout mice are corrected by the additional deletion of the other claudin.

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The tight junction (TJ) is a supramolecular structure forming strand-like connections between adjacent epithelial cells, thereby regulating paracellular permeability. The main constituent of the TJ strands are the claudins, which are tetraspanning membrane proteins that interact via their extracellular segments with claudins of the neighboring cells. Claudins are either sealing the paracellular cleft or forming a channel, thus conveying charge and size selectivity to the paracellular pathway.[1,2]

In humans, mutations in *CLDN16* cause the salt-wasting disorder familiar hypomagnesemia, hypercalciuria, and nephrocalcinosis (FHHNC), characterized by renal loss of Ca^{2+} and Mg^{2+}, and by nephrocalcinosis. In most cases, FHHNC leads to end-stage renal disease, ultimately requiring renal transplantation.[3] Patients with mutations in *CLDN10* present with anhidrosis and mild kidney failure in one family or with a hypokalemic-alkalotic salt-losing tubulopathy in another family.[4–6] Mice lacking claudin-16 display a pathology similar to that observed in FHHNC patients (i.e., hypomagnesemia and hypercalciuria).[7] In sharp contrast, mice specifically lacking claudin-10 in the same part of the nephron display hypermagnesemia, hypocalciuria, nephrocalcinosis, and polyuria.[8] Electrophysiological experiments in thick ascending limb (TAL) tubules lacking claudin-16 showed reduced paracellular Mg^{2+} and Ca^{2+} permeabilities.[7] TAL tubules of *Cldn10*-deficient mice showed a reduced paracellular sodium permeability paralleled by an increased Mg^{2+} and Ca^{2+} permeability, and a higher expression of claudin-16.[8]

On the molecular basis, claudin-10b forms paracellular cation channels, whereas permeability properties for mono- and divalent cations of claudin-16 alone or together with claudin-19 are still a matter of debate. The interaction of claudin-19 with claudin-16 is necessary for their proper localization to the TJs and their co-expression has been reported to increase the paracellular permeability to cations *in vitro*.[9,10] In contrast, expression of claudin-14 reduces the paracellular permeability to cations and acts as negative modulator of paracellular transport of divalent cations in response to elevated levels of Ca^{2+}.[11–13] In the kidney claudin-10b is highly expressed in ISOM (inner stripe of the outer medulla), where paracellular transport of Na^{+}

predominates, while claudin-16 is restricted to the OSOM and cortex, where it colocalizes with claudin-19.[14] Claudin-10 and claudin-16 thereby do not colocalize in the same TJ strands and form a mosaic pattern, the permselectivity for Na^{+} increasing with increasing percentage of claudin-10–positive TJ strands. Based on our previous findings in mice lacking either claudin-16 or claudin-10 in the TAL and their expression profile, we hypothesized that claudin-10 and claudin-16 indeed contribute to different paracellular channels with different permeabilities to Na^{+}, Ca^{2+} and Mg^{2+}.[8] To test this hypothesis and to provide the basis for a better understanding of FHHNC, we generated mice lacking both claudin-10 and claudin-16.

RESULTS

We generated a mouse model lacking claudin-10 and claudin-16 by crossing claudin-16–deficient mice (C16 KO)[7] with mice lacking claudin-10 specifically in the kidney (C10 cKO).[8] Resulting double-knockout mice (dKO) were born at Mendelian ratio, and were viable and fertile. We confirmed hypomagnesemia in C16 KO and hypermagnesemia in C10 cKO (Figure 1a). In contrast, in mice deficient for both claudins, serum Mg^{2+} concentration was in the range of control animals. Correspondingly, fractional excretion of Mg^{2+} (FE_{Mg}) was increased in C16 KO and decreased in C10 cKO, but normalized in dKO. While C16 KO mice showed a more than 3-fold increase in fractional excretion of Ca^{2+} (FE_{Ca}), dKO had urinary Ca^{2+} excretion levels comparable to controls (Figure 1b). Concomitantly, dKO showed no signs of nephrocalcinosis, a characteristic of hyperabsorption of divalent cations in C10 cKO (Figure 2).

The double-deficient mice exhibited polyuria that they compensated by increased water intake (Table 1). Urinary pH was lower in all mouse models compared with controls, ranging from a mild decrease in pH in C16 KO, to an intermediate acidity in the dKO, to a pronounced drop in urinary pH in C10 cKO mice. Na^{+}, Cl^{-} and K^{+} homeostasis was not affected in each of the single-KO animal models. Interestingly, dKO animals, if compared with C16 KO, developed a mild but significant drop in plasma K^{+}. Their increased FE_{K} was accompanied by a decrease in FE_{Na} indicating compensatory activity of the collecting duct to maintain salt balance.

Immunohistochemistry showed that claudin-16 and claudin-10 were differentially expressed along the corticomedullary axis in the TAL (Figure 3). Claudin-10 immunoreactivity was found in cortex and medulla in basolateral structures and in TJs. Claudin-16 was restricted to TJs of cortex and outer stripe of outer medulla (OSOM) and absent in the inner stripe of outer medulla (ISOM). The mosaic distribution of claudin-10 and -16 was clearly visible in sections from control animals. In the ISOM-only claudin-10 (Figure 3e,f), but in the cortex as well as in the OSOM, both claudins were expressed (Figure 3a,b). Whereas claudin-10 distribution remained unaltered in C16 KO (Figure 3d,h), claudin-16 expression extended to the ISOM in C10 cKO (Figure 3g). Both claudins were absent in dKO (data not shown).

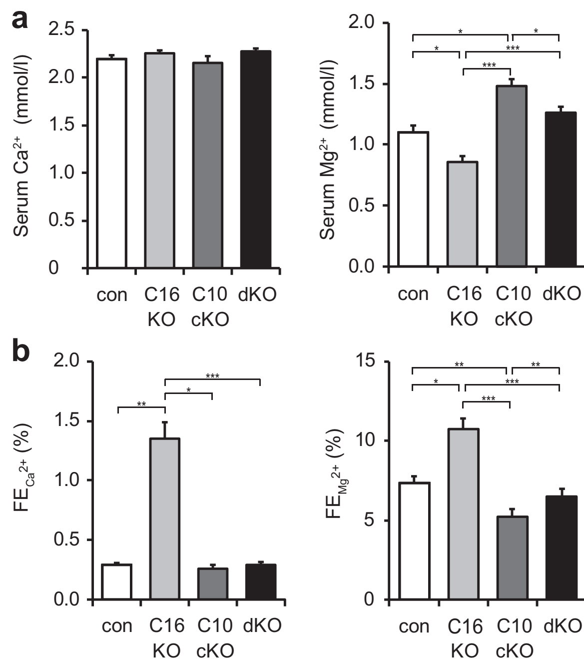

Figure 1 | Homeostasis of Ca^{2+} and Mg^{2+}. Summarized data are shown for serum (**a**) Ca^{2+} and Mg^{2+} concentrations and (**b**) fractional excretions (FE) of these divalent ions. In contrast to claudin-16 (C16) knockout (KO) (hypomagnesemia) and mice lacking claudin-10 specifically in the kidney (C10 cKO) (hypermagnesemia), double-knockout (dKO) mice show normalized Mg^{2+} concentrations. The hypercalciuria of C16 KO (shown as increased FECa) is normalized in dKO. Data are shown as mean ± SEM, $n = 5–11$. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ in 1-way analysis of variance with Holm-Bonferroni *post hoc* test.

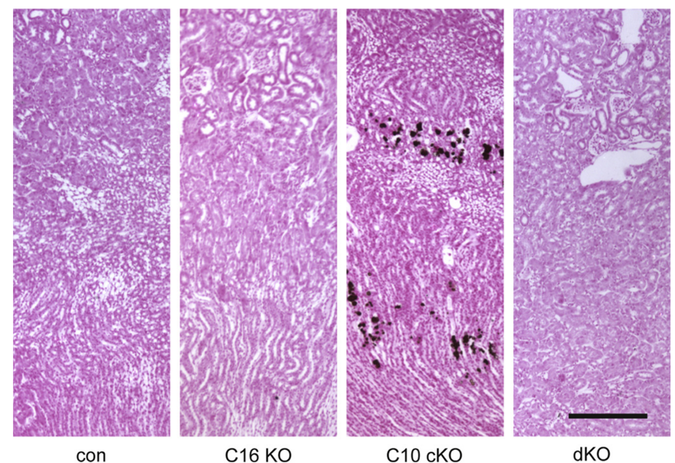

Figure 2 | Absence of nephrocalcinosis in kidneys of double-knockout (dKO). Strong von Kossa staining of Ca^{2+} precipitates is only found in kidney sections from mice lacking claudin-10 specifically in kidney (C10 cKO) mice but neither in claudin-16 knockout (C16 KO) nor in dKO. Sections were counterstained with eosin. Bar = 1 mm. con, control. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

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