The Role of Tight Junctions in Paracellular Ion Transport in the Renal Tubule: Lessons Learned From a Rare Inherited Tubular Disorder

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Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) is an autosomal recessive renal tubular disorder that typically presents with disturbances in magnesium and calcium homeostasis, recurrent urinary tract infections, and polyuria and/or polydipsia. Patients with FHHNC have high risk of the development of chronic kidney disease and end-stage renal disease in early adolescence. Multiple distinct mutations in the CLDN16 gene, which encodes a tight junction protein, have been found responsible for this disorder. In addition, mutations in another member of the claudin family, CLDN19, were identified in a subset of patients with FHHNC with visual impairment. The claudins belong to the family of tight junction proteins that define the intercellular space between adjacent endo- and epithelial cells. Claudins are especially important for the regulation of paracellular ion permeability. We describe a Brazilian family with 2 affected siblings presenting with the typical FHHNC phenotype with ocular anomalies. The clinical diagnosis of FHHNC was confirmed using mutational analysis of the CLDN19 gene, which showed 2 compound heterozygous mutations. In the context of the case vignette, we summarize the clinical presentation, diagnostic criteria, and therapeutic options for patients with FHHNC. We also review recent advances in understanding the electrophysiologic function of claudin-16 and -19 in the thick ascending limb of the loop of Henle and implications for ion homeostasis in the human body.


INDEX WORDS: Hypomagnesemia; nephrocalcinosis; claudin; tight junction; CLDN16; CLDN19.

BACKGROUND

Ion transport processes along the kidney tubule have a pivotal role in the homeostasis of serum electrolytes and maintenance of blood pressure in the human body. Transport modalities vary substantially between different nephron segments. Reabsorption of ions and water can be carried out by either active transport through an epithelial cell or passively through the paracellular pathway. Paracellular transport depends on specific characteristics of an epithelial cell layer in which adjacent cells are connected by specialized cell-to-cell contacts, the so-called tight junctions. These tight junctions may either seal the intercellular space or allow selective transport of charged solutes and water.

The importance of paracellular ion transport processes for electrolyte reabsorption in the kidney was emphasized by the discovery of mutations in 2 members of the claudin multigene family, CLDN16 and CLDN19, in patients with hereditary renal calcium and magnesium wasting because the claudin proteins have been shown to confer ion selectivity to tight junctions.

CASE VIGNETTE

A 19-year-old woman was first admitted to a primary care hospital for uremic symptoms. She was referred to the Pediatric Nephrology Department for further investigations and initiation of renal replacement therapy. She had an extensive medical history of multiple distinct renal tubular disorders: recurrent urinary tract infections, and retinitis pigmentosa was diagnosed at 2 years of age. Physical examination revealed visual impairment and horizontal nystagmus, and ophthalmologic examination showed severe bilateral retinopathy with macular coloboma.

Preliminary laboratory investigations confirmed end-stage renal failure (estimated glomerular filtration rate [GFR], 4 mL/min/1.73 m² [0.07 mL/min/1.73 m²] calculated using the Schwartz equation) and minor electrolyte disturbances: serum calcium level was within the reference range at 9.0 mg/dL (2.25 mmol/L), but in light of advanced chronic kidney disease (CKD), serum magnesium level was low at 1.38 mEq/L (0.69 mmol/L). Plain radiographs of the abdomen showed bilateral renal calcifications and nephrolithiasis (Fig 1A). Renal ultrasound showed bilateral medullary nephrocalcinosis and small kidneys (Fig 1B). Additional clinical and biochemical data are listed in Table 1.

Assessment of family history showed that retinitis pigmentosa also was diagnosed in the patient’s 13-year-old sister in early childhood, and she was almost blind. Funduscopy showed severe macular coloboma (Fig 1C and D). In addition, repeated...
episodes of cerebral convulsions of unknown origin were noted in the past. Renal ultrasound also showed severe medullary nephrocalcinosis, and laboratory investigations showed persistent hypomagnesemia caused by renal magnesium wasting (serum magnesium, 0.5 mEq/L [0.25 mmol/L]), hyperuricemia, and hypercalciuria (Table 1). As in the index case, urine sediment was compatible with a chronic urinary tract infection. Given an estimated GFR of 45 mL/min/1.73 m² [0.75 mL/s/1.73 m²], her level of kidney function was classified as CKD stage 3.

Clinical workup of the parents and the 16-year-old brother showed unremarkable findings.

Based on the key clinical and laboratory findings in the 2 sisters (hypomagnesemia, hypercalciuria, nephrocalcinosis, and kidney failure), the diagnosis of familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC; with eye involvement) was made. Genetic testing was initiated to confirm the clinical diagnosis in this family (discussed later).

**PATHOGENESIS**

**Familial Hypomagnesemia With Hypercalciuria and Nephrocalcinosis**

The first clinical description of FHHNC dates back to 1972 when Michelis et al.² first described a family affected with a hypomagnesemic disorder characterized by excessive renal magnesium and calcium wasting (Michelis-Castrillo syndrome). In addition, the patients had medullary nephrocalcinosis and developed CKD. Subsequent case reports and small series of patients enabled phenotypic characterization of the entire clinical spectrum of FHHNC, allowing differentiation from other hypomagnesemic disease states.³⁻⁷ The most frequent clinical symptoms at presentation are recurrent urinary tract infections and polyuria and/or polydipsia, which mostly occur between early childhood and school age.³⁻⁷ Other leading symptoms at presentation include kidney stone formation, failure to thrive, vomiting, abdominal pain, tetanic episodes, or generalized seizures. In addition to hypomagnesemia with a magnesium level of ~1 mEq/L (0.5 mmol/L), biochemical abnormalities include renal magnesium and calcium wasting; kidney function often is already impaired at the age of diagnosis. Serum intact parathyroid hormone levels tend to be increased before the stage of advanced CKD. Other laboratory findings may include incomplete distal renal tubular acidosis, hypocitraturia, and hyperuricemia. Extrarenal manifestations, mainly eye involvement, have been reported repeatedly. Visual impair-
ment may be very disabling in patients with FHHNC and is due to severe myopia, progressive retinopathy with the development of macular coloboma, and nystagmus.4,6,7

One third of the patients with FHHNC develop kidney failure before adolescence.8 Because a close correlation between progression of kidney failure and degree of nephrocalcinosis has been reported, therapeutic approaches aim at decreasing urinary calcium excretion.6 A short-term clinical study proved that treatment with thiazides decreases urinary calcium excretion in patients with FHHNC.9 However, to date, it is still unclear whether thiazides truly have the potential to attenuate the progression of GFR loss in patients with FHHNC.

A second therapeutic approach aims at preventing the secondary effects of hypomagnesemia using magnesium supplementation. As with progressive CKD, less magnesium can be filtered and thus excreted; hence, magnesium levels tend to increase with decreased kidney function.

As expected, recurrence of FHHNC after kidney transplant has never been observed because the primary defect resides in the kidney.

From the disease phenotype and clearance studies in patients with FHHNC, Rodriguez-Soriano et al4 postulated that the primary defect must be related to disturbances in paracellular magnesium and calcium reabsorption in the loop of Henle. Because no good candidate genes were available at that time, it was by using a pure positional cloning approach that Simon et al10 identified mutations in a novel gene (CLDN16 [formerly referred to as PCLN1]) on chromosome 3 as the underlying cause of FHHNC. CLDN16 encodes claudin 16 (paracellin 1), a tight junction protein of the claudin multigene family. To date, more than 40 different CLDN16 mutations have been identified in patients with FHHNC,8,10-16 and genotype-phenotype correlation suggests that the CLDN16 genotype may help predict the clinical course in affected individuals.14

There is evidence from family studies that carriers of heterozygous CLDN16 mutations also may present

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Note: Conversion factors for units: serum creatinine in mg/dL to μmol/L, × 88.4; serum urea nitrogen in mg/dL to mmol/L, × 0.357; uric acid in mg/dL to μmol/L, × 59.48; serum magnesium in mEq/L to mmol/L, × 0.5; serum calcium in mg/dL to mmol/L, × 0.2495; inorganic phosphorus in mg/dL to mmol/L, × 0.3229; eGFR in mL/min/1.73 m² to mL/s/1.73 m², × 0.01667. No conversion necessary for serum potassium, serum sodium, and bicarbonate in mEq/L and mmol/L and for PTH in pg/mL and ng/L.

Abbreviations: eGFR, estimated glomerular filtration rate; FE₉₀, fractional excretion of magnesium; FE₉₀, fractional excretion of sodium; ND, not determined; PTH, parathyroid hormone.
with clinical symptoms. Two studies described a high incidence of hypercalciuria, nephrolithiasis, and/or nephrocalcinosis in first-degree relatives of patients with FHHNC,6,8 and a subsequent smaller study reported a tendency toward mild hypomagnesemia.13 Also, analysis of a Cldn16 knockout mouse model showed that heterozygous Cldn16+/− mice exhibited a tendency toward hypercalciuria and increased 1,25-dihydroxyvitamin D3 levels, indicating the presence of an intermediate phenotype, as observed in heterozygous CLDN16 mutation carriers.17

Homozgous or compound heterozygous mutations in CLDN16 do not uniformly result in FHHNC. Muller et al18 described a homozygous threonine to arginine substitution at amino acid 303 (T303R; located in the carboxy-terminal PDZ binding motif [the PDZ stands for 3 proteins in which it initially was described, ie, postsynaptic density 95, discs large, and zonula occludens-1 (ZO-1)]) in 2 families with isolated hypercalciuria and nephrocalcinosis without evidence for a disturbance in renal magnesium handling. Interestingly, hypercalciuria disappeared during follow-up, and urinary calcium excretion normalized after puberty.18

FHHNC is a genetically heterogenous disorder because mutations in CLDN19, another member of the claudin gene family, also have been identified.19 The renal phenotype of patients with FHHNC with mutations in either CLDN16 or CLDN19 is virtually undistinguishable. However, clinical presentations differ in terms of the ocular phenotype because most patients with CLDN19 defects show severe visual impairment, whereas patients with CLDN16 mutations have no or only minor ocular abnormalities.

Consequently, to characterize the molecular defect in the family described in this review (see case vignette), direct sequencing of CLDN19 was performed. In both affected sisters, 2 heterozygous missense mutations in CLDN19 were identified: a glycine to aspartate substitution at amino acid 20 and a proline to leucine substitution at residue 28 (G20D and P28L, respectively; Fig 2A). The mother, who originated from Portugal, has inherited the G20D mutation, previously described as a Hispanic founder mutation.19 The father (mixed African-Brazilian ethnicity) carries the P28L mutation. This new missense mutation is located in the first transmembrane domain of claudin 19 (Fig 2B).

In the past, the deterioration in kidney function in patients with FHHNC associated with progressive tubulointerstitial nephropathy and end-stage renal disease in early adolescence was attributed to hypercalciuria and nephrocalcinosis.6 However, other inherited tubular disorders associated with severe medullary nephrocalcinosis do not uniformly lead to progressive CKD. For example, in antenatal Bartter syndrome, GFR is stable during the first 2 decades of life.20 This discrepancy indicates that severe nephrocalcinosis alone is not sufficient to explain the high rate of kidney failure in patients with FHHNC. From a clinical point of view, the repeated episodes of febrile urinary tract infections may be an additional risk factor for CKD in patients with FHHNC. As an alternative explanation, loss of kidney function might be related to the gene defect itself. This hypothesis is supported by the naturally occurring bovine claudin 16 knockout phenotype (complete deletion of Cldn16), which is characterized by early onset of end-stage renal disease.

**Figure 2.** (A) Family pedigree. Two heterozygous missense mutations in CLDN19 were identified in the 2 sisters (II.1 and II.3). Black bars indicate the G20D (glycine to aspartate substitution at amino acid 20) mutant allele; grey bars, the P28L (proline to leucine change at position 28) mutant allele; and white bars, the wild-type allele (WT). (B) Schematic model of claudin 19. Arrows indicate amino acid changes caused by the different mutations. Q57E, glutamine to glutamate substitution at amino acid 57; L90P, leucine to proline substitution at amino acid 90; G123R, glycine to arginine substitution at amino acid 123.
renal disease associated with interstitial nephritis and diffuse fibrosis. The established genotype-phenotype correlation for CLDN16 also is consistent with this explanation, given that a more rapid decrease in GFR is seen in patients affected by severe mutations that lead to complete loss of function compared with those with mutations in claudin 16 that preserve residual function.

Physiologic Principles: Ion Handling in the Thick Ascending Limb

Magnesium Homeostasis

Magnesium has an important role in the regulation of membrane transporters, signal transduction and as a cofactor for a great variety of enzymes. Under physiologic conditions, serum magnesium levels are maintained at almost constant values by up- or downregulation of intestinal absorption and renal excretion.

Approximately 80% of total serum magnesium is filtered in glomeruli. More than 95% of the filtrated magnesium then is reabsorbed along the different nephron segments, and only 3%-5% is excreted in urine. Whereas the proximal tubule accounts for only a small percentage of magnesium reabsorption, in terms of quantity, the thick ascending limb of the loop of Henle is the most important segment, in which ~70% of the filtered magnesium is reabsorbed. However, the final fine-tuning of magnesium excretion is carried out in the distal convoluted tubule, where different hormonal controls act. Magnesium transport in the thick ascending limb occurs mainly through the paracellular pathway, with the lumen-positive potential within the thick ascending limb as the driving force. Magnesium reabsorption in the distal convoluted tubule occurs actively through the transcellular pathway.

Paracellular Transport in the Thick Ascending Limb

To understand the pathophysiologic process of FHHNC, it is important to appreciate the principles of ion transport in the thick ascending limb (Fig 3). The thick ascending limb has 2 main functions: (1) absorption of sodium chloride, magnesium, and calcium; and (2) generation of an osmotic gradient as a prerequisite for water reabsorption in the following nephron segments. Sodium reabsorption occurs through 2 different pathways: an active transcellular transport and a passive paracellular route. Transcellular transport is mediated by the sodium-potassium-chloride (Na\(^+\)-K\(^+\)-2Cl\(^-\)) cotransporter 2 (NKCC2; encoded by the SLC12A1 gene) of Henle.
in the apical membrane of thick ascending limb cells and the basolateral adenosine triphosphatase sodium-potassium pump (Na\(^+\)-K\(^+\)-ATPase). At the same time, passive paracellular sodium reabsorption occurs through specialized tight junctions driven by the lumen-positive potential. Active sodium transport also provides the driving force for potassium and chloride uptake through the apical NKCC2. Whereas chloride ions leave the cell through chloride channels (CIC-Ka and CIC-Kb) at the basolateral side into the interstitium, only a small amount of the potassium eventually is reabsorbed through potassium channels at the basolateral membrane. Instead, most potassium is recycled back into urine through specific potassium channels (ROMK [renal outer medullary K\(^+\) channel]; encoded by the KCNJ1 gene) in the apical membrane of thick ascending limb cells (Fig 3). This recycling enforces the lumen-positive potential within the thick ascending limb and thus the driving force for paracellular reabsorption of other cations, especially magnesium and calcium.

As shown here, paracellular transport of cations in the thick ascending limb is dependent on paracellular cation selectivity and driving force. The former is provided by a specific composition of tight junction strands in the thick ascending limb involving expression of different claudins.

Along the axis of the thick ascending limb, the lumen-positive potential increases constantly. At the end of the thick ascending limb, the produced sodium gradient leads to paracellular backflow of sodium through the tight junction network. This in turn leads to a further increment in lumen-positive potential and therefore provides an additional driving force for the exclusive paracellular transport of magnesium and calcium.23,24

**RECENT ADVANCES**

**Claudin Physiology and Pathophysiology of FHHNC**

**tight Junction**

The tight junctions are located in the apical part of the lateral cell membrane of epithelial and endothelial cells, where they embrace the cells like a belt. The belt of one cell adjoins the belts of adjacent cells and the proteins interact with each other. Tight junctions serve as diffusion barriers for the transport of water and electrolytes and at the same time are able to induce specific paracellular permeability for ions or other solutes.23 In addition, tight junctions also ensure cell polarity.

Important components of the multiprotein tight junction complexes are the transmembrane proteins occludin,26 junctional adhesion molecules,27 tricellulin,28 and the protein family of claudins.29 According to the requirements of different epithelia, a specific composition of tight junction proteins can be found.25

**The Claudin Gene Family**

Since the discovery in 1998 of claudin 1 and 2 by Furuse et al.,29 22 additional members of this multi-gene family have been identified in humans. The molecular weight of the encoded proteins varies from 20-27 kDa.29,30 All claudins consist of 4 transmembrane helices, 2 extracellular loops, and intracellular amino and carboxy termini. Although the first loop is believed to influence paracellular charge selectivity, the second loop probably is responsible for the interaction between opposing claudins.

The carboxy terminus of most claudins contains a sequence known as the PDZ-binding motif.31 Given that the PDZ domain is found in the ZO-1 protein, claudins are capable of interacting with this most important member of the ZO proteins, thus anchoring with the actin cytoskeleton of the cell.31 All claudins also share the highly conserved amino acid sequence motif W-GLW-C-C (ie, tryptophan-glycine-leucine-tryptophan-cysteine-cysteine) in the first extracellular loop.

The extracellular loops are essential contact points for the formation of tight junction strands.32 Several claudins cannot only provide homotypic interaction with identical claudins, but also can establish heterotypic interactions with different claudins. However, not every theoretical combination is possible.33

The nomenclature claudin (from the Latin claudere, “to close”) refers to the first detected claudins, which show a sealing function.29 Further investigations showed that the paracellular permeability of different epithelia is modulated not only by up- or downregulation of the expressed sealing claudins,34 but also by alteration in the expression of pore-forming claudins.35

Some claudins show a broad expression pattern, whereas others are expressed specifically in a certain subset of tissues. In the kidney, there is a segment-specific claudin expression profile along the nephron, according to the different functional requirements of each nephron segment. In a murine kidney extract, all claudins except 6, 9, 13, and 14 can be detected using Northern blot probing of messenger RNA expression.36 Claudins 1 and 2 could be identified in the Bowman capsule; claudins 2, 10, and 11, in the proximal tubule; and claudins 3, 7, 8, and 16, in the distal tubule. Claudins 2, 3, 4, and 8 were found in the thin ascending limb of the loop of Henle; claudins 3, 10, 11, 16, and 19, in the thick ascending limb of the loop of Henle; and claudins 1, 3, 4, 8, and 10, in the collecting duct.10,19,36-38

Biology and Physiology of Claudin 16

Claudin 16 is expressed mainly in thick ascending limb cells. The gene encoding human claudin 16 has 2 in-frame start codons; the first produces a 305-amino acid protein, and the second, which occurs at the methionine at position 71, corresponds to the start codon of mouse and rat Cldn16.35 The downstream sequence of these 2 orthologues is highly conserved. Using immunofluorescence imaging of mammalian cell lines expressing human CLDN16 complementary DNA, Hou et al40 showed that the short claudin 16 variant concentrated at the tight junction, whereas the long claudin 16 variant was restrained in endosomes or lysosomes. The physiologic relevance of the second translation start site also is supported by the observation that genetic analysis of patients with FHHNC has uncovered a point mutation leading to the substitution of methionine by threonine at amino acid 71 (ie, a mutation that disrupts the start codon) that is linked to the disease and causes mistargeting of claudin 16 to lysosomes.14 In line with these results, a common insertion-deletion polymorphism at position 55 of the long isoform can be identified in healthy humans.8 This variant leads to a frame shift and truncation of the long isoform of the protein at a premature stop codon at position 90; however, because it occurs in the untranslated region, it does not affect the short isoform.

As mentioned, mutations in the human CLDN16 gene cause a selective disturbance in renal magnesium and calcium reabsorption in the thick ascending limb.13 The current hypothesis is based on the idea that the unusually high number of negatively charged amino acids in the first extracellular loop may allow the formation of a cation-selective filter and thereby enable the absorption of divalent cations. Most mutations are located within the first extracellular loop.

Electrophysiologic Function of Claudin 16

Different electrophysiologic studies have investigated the underlying mechanism of the paracellular transport of ions through a tight junction formed by claudin 16. Ikari et al31 transfected claudin 16 in low-resistance MDCK (Madin-Darby canine kidney) cell monolayers and reported increased calcium flux from the apical to basolateral direction, but not the other way around. In contrast, magnesium flux remained equal to control cells. However, overexpression of claudin 16 transfected in high-resistance MDCK cells was able to increase paracellular magnesium permeability, although this increase turned out to be moderate.11

Finally, transfection of claudin 16 in anion-selective LLC-PK1 cells, a porcine kidney line, showed a significant increase in sodium permeability with only a moderate increase in magnesium permeability.40 The increase in paracellular cation permeability seems to be an important physiologic function of claudin 16 because it could not be detected in all claudin 16 mutants responsible for FHHNC investigated40 and connects claudin 16 directly to the development of the electrical transepithelial gradient that serves as the driving force for the reabsorption of cations, especially magnesium and calcium, in the thick ascending limb.

Taken together, all published investigations concerning the electrophysiologic function of claudin 16 in different cell types could detect only a marginal increase, if any, in paracellular magnesium transport and calcium permeability after overexpression of claudin 16. Interestingly, none of the detected mutations to date concern the negatively charged amino acids in the first extracellular loop challenging the mentioned hypothesis.

Claudin 16 Mouse Models

To date, 2 different mouse models are available. The claudin 16 knockdown mouse generated by RNA interference (small interfering RNA [siRNA]) technology shows hypomagnesemia and significantly increased urinary excretion of magnesium and calcium. Renal histologic examination showed calcium deposits along the basement membranes of medullary tubules, as well as in the interstitium, similar to findings observed in humans with FHHNC.42 Studies concerning electrophysiologic function in the thick ascending limb by perfusion of isolated thick ascending limb segments showed a decrease in transepithelial potential. This decrease consequently leads to an inability to reabsorb cations through the paracellular pathway; thus, urinary excretion of these cations is increased.42

Recently, a Cldn16 knockout mouse model was generated. In accordance with the human phenotype, knockout mice (Cldn16−/−) show hypomagnesemia and hypercalciuria, as well as compensatory pathways, such as increased parathyroid hormone and 1,25 dihydroxyvitamin D3, similar to observations in patients with FHHNC.17 Interestingly, juvenile Cldn16 knockout mice had normal urinary excretion values for magnesium, sodium, and potassium.17

In contrast to claudin 16 knockdown mice and the human disease phenotype, Cldn16 knockout animals did not develop renal calcifications. The investigators proposed the presence of a specific compensatory mechanism in knockout mice preventing renal calcification.17 Interestingly, transcriptional upregulation of other transport systems for divalent cations, including Trpv5, Trpm6, and Calbindin-D9k, was observed.17 Both animal models did not develop CKD.
Electrophysiologic studies of perfused thick ascending limb tubules of 9- to 10-week-old Cldn16−/− knockout mice showed no alteration in paracellular permeability for monovalent cations, such as sodium or lithium. However, for magnesium and calcium, a significant decrease in paracellular permeability could be detected.17

In conclusion, the available data indicate that claudin 16 confers nonselective paracellular cation permeability to tight junction strands of the thick ascending limb.

**Biology and Physiology of Claudin 19**

Claudin 19 is expressed in various tissues, with the highest expression in the kidney and eye. In the peripheral nervous system, claudin 19 forms tight junctions resembling structures in Swann cells.43

In kidney tubules, claudin 16 and 19 share similar expression patterns: reverse-transcription polymerase chain reaction assays in microdissected mouse nephron segments could detect expression of both Claudins in medullary and cortical thick ascending limbs, as well as in the distal convoluted tubule.19 This nephron segment-specific colocalization implies that claudin 16 and 19 share a similar function.

Patients presenting with typical FHHNC symptoms, but without a detectable mutation in the CLDN16 gene, therefore were tested for mutations in CLDN19. In addition to the known phenotype, they showed severe ocular involvement with macula coloboma, myopia, and horizontal nystagmus, as mentioned.19 In the eye, claudin 19 is expressed specifically in the retina, with strong expression in the retinal pigment epithelium, inner plexiform, and inner nuclear layer.19 Notably, claudin 16 is not expressed in the retina body, which may explain the lack of ocular defects in patients with FHHNC with CLDN16 mutations. A proper tight junction formation is required for cell polarity and epithelial barrier function. Thus, loss of correct tight junction formation can result in mistargeting of factors influencing retinal cell division, leading to the observed ocular phenotype.19

To date, 5 missense mutations affecting different parts of the claudin 19 protein have been published (Fig 2B), all of which involve the transmembrane domains or the first extracellular loop.19,23

**Electrophysiologic Function of Claudin 19**

Based on transfection experiments in LLC-PK1 cells reported by Hou et al.,23 claudin 19 leads to a significant decrease in chloride permeability without affecting sodium permeability. All CLDN19 mutations causing FHHNC in humans (G20D, Q57E [glutamine to glutamate change], L90P [leucine to proline], and G123R [glycine to arginine]) resulted in loss of this function. Interestingly, no effect of claudin 19 could be found on permeability for magnesium and sodium.23

Angelow et al14 analyzed MDCK cells transfected with claudin 19 and found a significant decrease in paracellular permeability for monovalent and divalent cations.

All these observations together indicate a complex function of claudin 19, rather than simply the formation of paracellular pores for divalent cations, as initially assumed. However, it has to be kept in mind that data for claudin 19 and 16 function were generated mainly in overexpression systems disturbing the physiologic composition of tight junction strands in polarized cell lines. Moreover, different cell lines differ greatly in background levels of claudin expression.

**Claudin 19 Mouse Model**

On account of the presence of claudin 19 in Swann cells, Cldn19 knockout mice show abnormal behavior and peripheral neuropathy, which correlated with dysfunctional tight junctions in Swann cells. Unfortunately, no data were published concerning the renal phenotype of Cldn19 knockout mice.43

Recently, Hou et al.45 created a claudin 19 knockdown mouse by using siRNA. These claudin 19 knockdown mice show a phenotype similar to the claudin 16 knockdown mice and patients with FHHNC. Biochemical analysis showed decreased magnesium and calcium plasma levels and increased urinary excretion of these divalent cations. No changes in plasma or urinary sodium levels could be detected. In addition, these mice showed increased aldosterone plasma levels, which presumably prevent sodium loss by activating the renin-angiotensin-aldosterone system and thus increasing sodium reabsorption in distal nephron segments. This hypothesis is supported by the observation of decreased potassium levels in plasma and increased urinary potassium excretion. In summary, the claudin 19 knockdown mice showed renal magnesium and calcium wasting due to defective paracellular cation permeability.45

**Interaction Between Claudin 16 and Claudin 19**

As mentioned, Claudins can interact with each other in 2 ways: within the cell membrane of the same cell (also called cis-interaction) or between the cell membranes of 2 adjacent cells (trans-interaction)33,46 (Fig 4). If mouse L fibroblasts, which normally do not form tight junctions, are transfected with both Claudins, well-developed tight junction strands with claudin 16 and 19 can be observed. Different missense mutations in either CLDN19 (L90P and G123R) or CLDN16 (L145P [leucine to proline], L151F [leucine to phenylalanine], G191R [glycine to arginine], A209T [alanine to threonine], and
F232C [phenylalanine to cysteine]) that cause FHHNC lead to a loss of interaction between claudin 16 and claudin 19 (heteromeric interaction), thereby disrupting the synergistic effect.\(^{23,45}\)

As published recently, analysis of claudin 16 and 19 knockdown mice (using siRNA) showed that loss of claudin 16 or 19 expression prevents the respective interacting claudin from reaching the tight junction.\(^{45}\) This observation again emphasizes the strong connection between these 2 claudins. Of interest, in that analysis of Cldn16 knockout mice, significantly lower claudin 19 expression was observed.\(^{17}\) Expression decreases with age and was decreased by 30% in adult compared with neonatal mice. Maybe the late-onset of hypomagnesemia in the juvenile Cldn16 knockout animals is triggered by the decrease in claudin 19 levels during development,\(^{17}\) which supports the importance of the claudin 16 and claudin 19 interaction.

**SUMMARY**

FHHNC is an autosomal recessive renal tubular disorder characterized by urinary calcium and magnesium wasting. Affected individuals have a high risk of the development of CKD, and extrarenal symptoms include severe visual impairment in a subset of patients. FHHNC is caused by mutations in 2 genes coding for tight junction proteins of the claudin multi-gene family, claudins 16 and 19. In the kidney, both proteins are expressed predominantly in the thick ascending limb of the loop of Henle, the major site of passive paracellular reabsorption of divalent cations. Functional data from overexpression studies in epithelial cell monolayers point to an important function of claudin 16 in conferring paracellular cation permeability, whereas claudin 19 prevents the paracellular flux of chloride. Genetic analyses and subsequent functional studies of mutated claudin 16 and 19 in this rare disorder show the highly specialized tissue-specific nature of tight junction composition and emphasize the critical role of claudins in selectively permitting or inhibiting paracellular ion flux along the kidney tubule. In the future, claudins and tight junctions eventually may provide targets for pharmacologic intervention. However, our present knowledge of the tight

**Figure 4.** Schematic model of claudin (Cldn) 16 and 19 interactions. (A) The term *cis*-interaction describes the interaction between claudins in the same cell membrane, whereas *trans*-interaction stands for the interaction between claudins located in adjacent cells. Claudin 19 is capable of interacting in a *cis*- or *trans*-manner with itself (homomeric interaction); homomeric interaction has not been observed for claudin 16. (B) There is no evidence of a direct interaction between claudin 16 and 19 in the *trans*-formation. (C) Consequently, a 3-stage hypothesis of tight junction assembly arises: (i) claudin 16 *cis*-interacts with claudin 19 (heteromeric) in one cell membrane, (ii) claudin 19 *trans*-interacts with itself (homomeric) between the cell membranes of adjacent cells, and (iii) claudin 16 and 19 again *cis*-interact in the adjacent cell membrane, finally creating the observed tight junction strands. Data source: Hou et al\(^{23}\) and Hou et al.\(^{45}\)
juncture physiologic process is still incomplete, and much work remains to be done before we could even think about successful therapeutic strategies for FH-HNC.

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