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Exceptional Case

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Two novel mutations of the *CLDN16* gene cause familial hypomagnesaemia with hypercalciuria and nephrocalcinosis

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Abstract
Familial hypomagnesaemia with hypercalciuria and nephrocalcinosis is an autosomal-recessive disease caused by mutations in the *CLDN16* or *CLDN19* genes, which encode tight junction-associated proteins, claudin-16 and -19. The resultant tubulopathy leads to urinary loss of Mg^{2+} and Ca^{2+}, with subsequent nephrocalcinosis and end-stage renal disease (ESRD). An 18-year-old boy presented with chronic kidney disease and proteinuria, as well as hypomagnesaemia, hypercalciuria and nephrocalcinosis. A kidney biopsy revealed tubular atrophy, interstitial fibrosis and segmental sclerosis of some glomeruli. Two novel mutations in the *CLDN16* gene were identified: c.340C>T (nonsense) and c.427+5G>A (splice site). The patient reached ESRD at 23 and benefited from kidney transplantation.

Keywords: claudin-16; nephrocalcinosis; proteinuria

Background

Familial hypomagnesaemia with hypercalciuria and nephrocalcinosis (FHHNC, OMIM #248250) is a rare autosomal-recessive renal tubular disorder caused by mutations in the *CLDN16* or *CLDN19* gene, which, respectively, encode the tight junction-associated proteins, claudin-16 and -19 [1–3]. These transmembrane proteins regulate the paracellular diffusion of selective cations along the thick ascending limb (TAL) of Henle's loop (Figure 1) [4, 5]. FHHNC-associated tubulopathy is thus characterized by massive urinary losses of Mg^{2+} and Ca^{2+}, with subsequent hypomagnesaemia, bilateral nephrocalcinosis and rapid evolution to end-stage renal disease (ESRD) [8]. More than 40 different mutations of the *CLDN16* gene have been described thus far. A genotype/phenotype correlation regarding the severity of the disease has been proposed upon the impact of *CLDN16* mutations on protein function [9]. Still, the pathophysiology of FHHNC remains unclear. We report on an 18-year-old patient presenting with glomerular proteinuria associated with the typical FHHNC triad. Further investigations demonstrated severe tubular atrophy and interstitial fibrosis, as well as secondary glomerulosclerosis. Two novel mutations of the *CLDN16* gene were identified.

Case report

The patient was referred for renal investigations after a fortuitous finding of increased serum creatinine levels (1.7 mg/dL), i.e. a glomerular filtration rate (GFR) of 52 mL/min per 1.73 m^2 according to the modification of diet in renal disease (MDRD) equation. His medical history included severe dehydration at birth, as well as persistent polyuria/polydipsia syndrome with nycturia since infanthood. No urinary tract infections or muscular cramps were reported. One year prior to consultation, he developed acute kidney injury in a context of rhabdomyolysis and dehydration in a motorcycle crash. At that time, bilateral non-complicated kidney stones were found. The patient's father was known for recurrent nephrolithiasis. Clinical examination was unremarkable. Eye inspection showed no abnormalities. Blood and urine parameters are summarized in Table 1. Note the co-occurrence of hypomagnesaemia, hypermagnesuria and hypercalciuria, with heavy selective proteinuria. Such abnormalities pointed to both tubular and glomerular dysfunctions. An oral glucose challenge test was normal. Pak's oral Ca^{2+} load test led to a significant decline in parathormone levels, thereby ruling out primary hyperparathyroidism and supporting a renal origin for hypercalciuria. Abdominal ultrasound disclosed symmetric 10-cm kidneys, with nephrocalcinosis and multiple millimetric lithiasis as confirmed by computed tomography. A kidney biopsy showed both atrophy and hypertrophy of renal tubules and interstitial fibrosis in association with focal and segmental sclerosis of glomeruli (Figure 2A and B). Von Kossa staining identified tubular Ca^{2+} deposits (Figure 2C). The expression of claudin-16 in TAL was lost, whereas the distribution of uromodulin did not appear to be significantly affected (Figure 2D–E).

Medical treatment included thiazides and angiotensin-converting enzyme (ACE) inhibitors, as well as oral

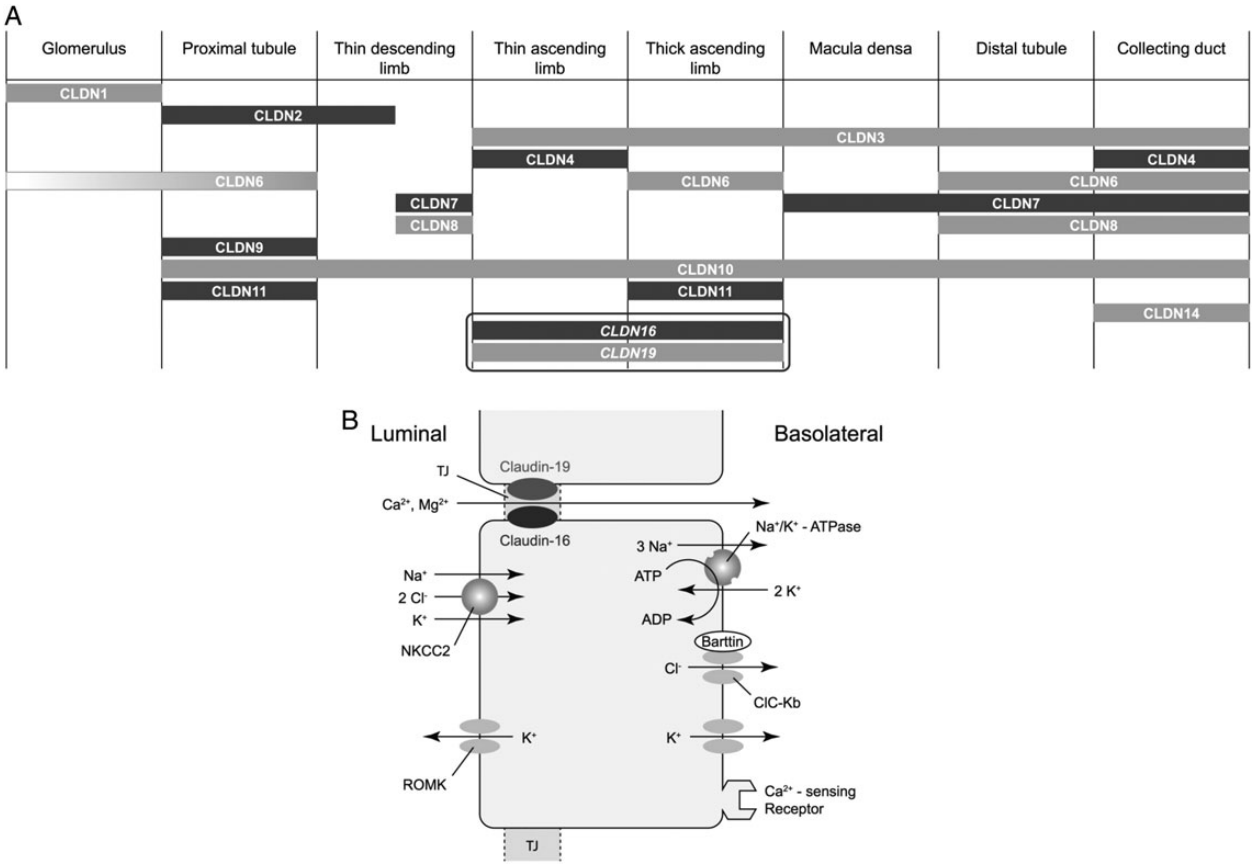

Fig. 1. Segmental and cellular distribution of claudin-16 and -19 along the mammalian nephron. (**A**) Localization of members of the claudin family in a mammalian kidney. Claudin-16 and -19 are specifically situated between epithelial cells lining the ascending limb of Henle's loop. Adapted from Angelow *et al.* [5]. (**B**) Schematic view of an epithelial cell lining the TAL of Henle's loop. Claudin-16 and -19 are positioned at the tight junctions (TJ) between adjacent cells, where they selectively regulate the paracellular diffusion of Ca^{2+} and Mg^{2+}. Ion transporters implicated in generating and/or modulating the positive trans epithelial electrical gradient, including Na^+/K^+-ATPase, ClC-Kb/barttin, Ca^{2+}-sensing receptor, ROMK (renal outer medullary K^+ channel) and NKCC2 (Type 2 $Na^+/K^+/Cl^-$ cotransporter) are depicted. Different K^+ channels from four gene families have been identified within the TAL basolateral membrane of various mammals. Adapted from Naderi and Reilly [6], Hamilton and Devor [7].

Table 1. Analysis of serum and 24-h urine samples at admission

			Normal values	
Serum	SI units	Conventional units	SI units	Conventional units
Creatinine	141.4	1.6	50–110 μmol/L	0.6–1.2 mg/dL
GFR	54		>60 mL/min per 1.73 m^2	
Uric acid	517.5	8.7	120–420 μmol/L	2–7 mg/dL
Magnesium	0.6	1.4	0.75–1 mmol/L	1.8–2.4 mg/dL
Calcium	2.3	9.2	2.1–2.6 mmol/L	8.4–10.6 mg/dL
Sodium	142	142	135–145 mmol/L	135–145 mEq/L
Potassium	3.2	3.2	3.1–4.9 mmol/L	3.1–4.9 mEq/L
Bicarbonate	33	33	23–33 mmol/L	23–33 mEq/L
Glucose	4.4	80	3.3–5.5 mmol/L	60–100 mg/dL
Intact PTH	298	298	12–58 ng/L	12–58 pg/mL
25-OH vitamin D	89.8	36	>80 nmol/L	>32 ng/mL
Urine				
Magnesium	8.5	17	3–4 mmol/day	6–8.5 mEq/day
Calcium/creatinine		0.29		0.04–0.15 g/mg
Proteins	1854	1.854	<150 mg/day	<0.150 g/day

GFR, glomerular filtration rate according to MDRD equation; PTH, parathyroid hormone.

supplementation of Mg^{2+} and active vitamin D. Still, despite the complete resolution of proteinuria under treatment, the patient reached ESRD at the age of 23. The slope of GFR decline was calculated to be 9 mL/min per 1.73 m^2/year. The patient pre-emptively benefited from a deceased donor kidney transplant. The 1-year follow-up showed an uneventful evolution, with a stable GFR ∼50 mL/min per 1.73 m^2. The pre-transplant work-up prompted genetic testing, which allowed the identification of two novel mutations in the *CLDN16* gene: c.340C>T and c.427+5G>A.

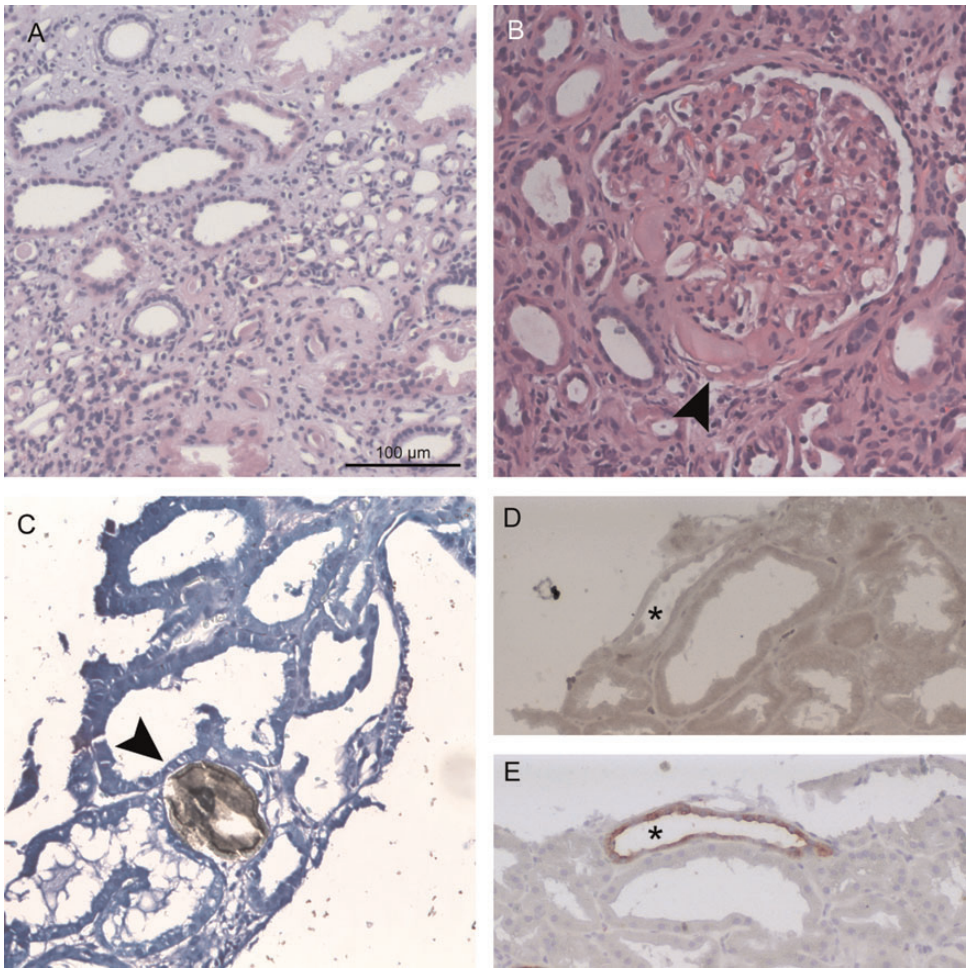

Fig. 2. Kidney histology of a patient with c.340C>T (nonsense) and c.427+5G>A (splice-site) mutations of *CLDN16* gene. Haematoxylin–eosin colouration (**A** and **B**) shows diffuse tubular atrophy and interstitial fibrosis, as well as perihilar segmental sclerosis of some glomeruli (B, arrowhead). Von Kossa staining (**C**) identifies intra-tubular Ca^{2+} deposits. Immunostaining anti-claudin-16 (**D**) and anti-uromodulin (**E**) on serial sections (cfr asterisk, *) does not detect claudin-16 (D) in uromodulin-positive tubules lining the TAL of Henle's loop (E). Scale bar: 100 µm (A–E).

The latter was also found in the patient's mother. His father, who presented with recurrent nephrolithiasis, could not be tested because of sudden death at the age of 52.

Discussion

The pathogenesis of chronic kidney disease (CKD) in FHHNC remains debatable [10]. Hypercalciuria and nephrocalcinosis most probably have a negative impact. Still, all inherited tubulopathies characterized by nephrocalcinosis do not uniformly lead to ESRD, which suggests that the functional loss of claudin-16 may *per se* impair kidney architecture and function [11]. Previous reports listed 40 missense/nonsense mutations, four splice-site mutations and 5 indels of the *CLDN16* gene [10]. Genotype/phenotype correlation studies postulated an earlier onset of the disease and a more rapid decline of GFR in patients harbouring *CLDN16* mutations inducing a complete loss of function of the protein in comparison with patients with mutations associated with a partial dysfunction [9]. Our patient presented at the age of 18 with Stage 3a CKD and significant selective proteinuria. Symptoms obviously developed in early infanthood, and the slope of GFR decline was >5 mL/min per 1.73 m^2/year, which suggests a complete loss of function of claudin-16 [9]. Kidney histology further supports an advanced and chronic tubulopathy, with tubular atrophy and Ca^{2+} deposits. In addition, sclerotic lesions were found in some glomeruli, with a particularly perihilar distribution (Figure 2). Such a location is suggestive of secondary glomerulosclerosis, and accounts for the glomerular proteinuria highly responsive to ACE inhibitor therapy. The complete loss of claudin-16, as demonstrated by immunostaining, may thus cause progressive nephron loss with tubular atrophy and interstitial fibrosis and secondary glomerular damage.

Two novel mutations were identified in a compound heterozygous state. The c.340C>T (p.R114*) mutation is a nonsense mutation located in exon 2, which most likely leads to a premature stop codon and a complete loss of claudin-16 function. This mutation is located in the first extracellular loop of the protein, similar to the large majority of previously reported mutations [10]. The c.427+5G>A mutation affects an intronic nucleotide located close to the donor splicing site of *CLDN16* exon 2. The pathogenic character of this mutation needs to be confirmed by expression studies. Still, given the severity of the disease and the absence of claudin-16 expression in the patient's TAL tubules, we speculate that this mutation also

results in a complete loss of claudin-16 function. Of note, a mutation with residual expression and function of the protein would predict a milder clinical course [9, 10].

In summary, FHHNC is a rare tubulopathy rapidly causing ESRD. In addition to the identification of novel mutations of *CLDN16* gene, the present case illustrates a late diagnosis of FHHNC in early adulthood and emphasizes the progressive nephron loss associated with this tubulopathy, as well as the glomerular consequences. There is no specific therapy for FHHNC. Conservative management of CKD and oral supplementation of Mg^{2+} remain the cornerstones of FHHNC treatment. The efficiency of thiazides, which decrease urinary Ca^{2+} excretion, on GFR decline remains controversial. Recurrence of FHHNC after kidney transplantation has never been observed.

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Conflict of interest statement. None declared.

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