# Hypomagnesemia-hypercalciuria-nephrocalcinosis and ocular findings: a new claudin-19 mutation

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**Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) is an autosomal recessive syndrome that affects the tight junction proteins claudin-16 and claudin-19 in the thick ascending limb. In patients with claudin-19 mutations, additional symptoms such as visual impairment and other ophthalmologic findings are expected.**

**In this report, we present a seven-year-old girl with polyuria and polydipsia. She was the daughter of consanguineous parents with a history of neonatal hypomagnesemic convulsion. On physical examination, bilateral horizontal nystagmus, retinitis pigmentosa and severe myopia were detected. Laboratory examination revealed hypomagnesemia, hypercalciuria and hypermagnesuria. A clinical diagnosis of FHHNC caused possibly by claudin-19 mutation was decided with the ocular findings. DNA analysis revealed a novel homozygous nonsense mutation (W169X) in the *CLDN19* gene.**

**In conclusion, in a patient with consanguineous parents, history of hypomagnesemic convulsion and disturbed organization and development of the retina, a diagnosis of FHHNC caused by claudin-19 mutation should be considered.**

***Key words: hypomagnesemia, hypercalciuria, nephrocalcinosis, retinitis pigmentosa, nystagmus, myopia, claudin-16, claudin-19.***

Inherited forms of renal hypomagnesemia are rare disorders. Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) is an autosomal recessively inherited form of renal magnesium transport disease[1].

In most cases, FHHNC is caused by loss-of-function mutations in the CLDN16 gene, which encodes claudin-16. In a subset of families with FHHNC with severe ocular involvement, the disease was shown to be caused by mutations in the CLDN19 gene, which encodes claudin-19[1]. In this report, we present a patient with hypomagnesemia, hypercalciuria, nephrocalcinosis, and ocular findings due to a new CLDN19 mutation (W169X), which was not reported previously. The aim of this report is to draw attention to the key clinical and laboratory findings for the differential diagnosis of FHHNC on clinical bases.

## Case Report

A seven-year-old girl was referred to our pediatric nephrology clinic for the evaluation of polyuria and polydipsia. The parents had recognized this symptom from the 6$^{th}$ month of age. Recently, she had been drinking 4.5 L and 1.5 L of water per day and night, respectively. Abdominal ultrasonography was performed because of complaints about bilateral costovertebral pain. Bilateral medullary nephrocalcinosis was detected before she was referred to our hospital.

The parents were cousins, and her postnatal history revealed hypocalcemic and hypomagnesemic convulsions on the 14$^{th}$ postnatal day. She was discharged with normal serum magnesium level. No convulsions had been recorded since then.

Her height was 122 cm (50$^{th}$ percentile), body weight 22 kg (25$^{th}$ percentile) and blood

pressure 109/70 mmHg (<90 percentile). Her physical examination revealed bilateral horizontal nystagmus. On ocular examination, visual acuities bilaterally were 20/100, and biomicroscopy was normal bilaterally. On fundus examination, around arcade vasculature and on the temporal side of the macula, hypertrophy of retinal pigment epithelium and bull's eye maculopathy were present.

Laboratory examination revealed: urine pH: 7, specific gravity: 1010; blood pH: 7.38, $HCO_3$: 19.7 mEq/L, serum calcium 7.3 mg/dl (8.4-10.2), phosphorus 4.3 mg/dl (2.7-4.5), magnesium 1.2 mg/dl (1.6-2.6), blood urea nitrogen (BUN) 14.6 mg/dl (7-25.7), creatinine 0.8 mg/dl (0.6-1.3), sodium 142 mEq/L (136-145), potassium 3.7 mEq/L (3.5-5.1), uric acid 4 mg/dl (2.6-7.2), parathyroid hormone (PTH) 466.7 pg/ml (15-65 pg/ml), urine calcium 12.2 mg/kg/day (<4/mg/kg/day), urine magnesium 3.37 mmol/day (N<0.46 mmol/day), and urine citrate 7.31 mmol/1.73 $m^2$/day (N= 0.7-4.6 mmol/1.73 $m^2$ /day).

The differential diagnosis of inherited forms of renal hypomagnesemia with polyuria, hypomagnesemia, hypercalciuria, nephrocalcinosis, and ocular findings revealed the possibility of claudin-19 mutation. DNA obtained from the blood was analyzed at University Children's Hospital, Münster, Germany, and revealed a novel homozygous nonsense mutation (p.W169X, c.G697A, TGG>TAG). She was commenced on magnesium citrate and thiazide diuretics.

**Discussion**

It is known that approximately 80% of total plasma magnesium is filtered by the glomeruli, and nearly 70% of this is reabsorbed by a passive paracellular mechanism in the thick ascending limb of Henle (TAL)[2]. Molecular identification of defects in claudin-16 and -19 in patients with FHHNC sheds light on the mechanism of the magnesium homeostasis in the TAL[3]. Claudins are major determinants of paracellular permeability in the epithelia. Cells of the TAL include an unusually large number of different claudins, within this claudin-16 and claudin-19[4]. CLDN16 and CLDN19 depleted tight junctions show normal barrier functions, but ion selectivity is defective[5]. In mouse models, it has been shown that siRNA knockdown of CLDN19 caused a loss of CLDN16 from tight junctions in the TAL without a decrease in the CLDN16 expression level, whereas siRNA knockdown of CLDN16 produced a similar effect on CLDN19. These data indicate that a heterodimeric claudin-16 and claudin-19 interaction was required for tight junction structure and cation-selective paracellular function[5]. Furthermore, in vitro studies showed that CLDN16/ CLDN19 interact at tight junction synergistically. CLDN16 functions as a $Na^+$ channel whereas CLDN19 functions as a $Cl^-$ blocker. When both CLDN16 and 19 are lost in the kidney, tight junction will become highly permeable to $Cl^-$ but not to $Na^+$, and no $Mg^{+2}$ can be reabsorbed. To date, 45 different (35 missense/ nonsense, 4 splicing, 3 small deletions, 2 small indels) claudin-16 mutations and five claudin-19 missense mutations have been reported (http://www.hgmd.cf.ac.uk)[6]. The five different reported mutations are localized in the first transmembrane domain (TMD) (2 mutations), first extracellular loop (ECL), second TMD, and third TMD of the claudin-19 protein, respectively. The mutations in the first TMD and first ECL result in complete loss of CLDN19 function, whereas the mutations in the second and third TMD result in partial loss of CLDN19 function[7]. Our patient's mutation is new, resulting in a preterminal stop codon leading to a shortened claudin-19 protein that lacks the C-terminal end.

Claudin-19 is expressed in the kidney, retina and myelinated peripheral neurons[3, 8, 9]. The renal phenotype of patients with FHHNC with mutations in either CLDN16 or 19 is virtually indistinguishable. However, clinical presentation differs in terms of ocular phenotype. Most patients with CLDN19 defects show severe visual impairment, whereas patients with CLDN16 mutations have no or only minor ocular abnormalities. There is a reported case of CLDN16 mutation, which was defined with additional congenital anomalies independent of the disease[10].

Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) patients usually present with recurrent urinary tract infection, polyuria, polydipsia, renal stones, and/or failure to thrive[1,11]. Our patient's first symptom was a neonatal convulsion that was associated with

hypocalcemia and hypomagnesemia. However, she was unfortunately treated with calcium and magnesium at that time and discharged with a normal magnesium value without a specific diagnosis. Obscure subnormal values of magnesium during infancy caused the delay in diagnosis. Thereafter, severe ocular abnormalities were the most significant symptom that forced the child's presentation for treatment. Polyuria and polydipsia together with costovertebral pain were the reference symptoms to our hospital. Severe depression of the serum magnesium did not inhibit increased urine magnesium excretion in these patients expectedly, because of defective ion selectivity in the tight junction[12]. Actually, it has been known that magnesium depletion itself may suppress PTH secretion, but in these patients, increased PTH levels were reported despite normal renal function[1]. Mild distal renal tubular acidosis has been reported previously and was also recorded in our patient[13]. Surprisingly, there was no history of recurrent urinary tract infection, which is one of the prominent symptoms of FHHNC. We speculate that the previously unreported new mutation (W169X) in our patient may explain the prevention of urinary tract infection.

In conclusion, in a patient with consanguineous parents, history of hypomagnesemia convulsion and disturbed organization and development of the retina, a diagnosis of FHHNC caused by CLDN19 mutation should be considered.

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