Familial hypomagnesemia with hypercalciuria and nephrocalcinosis in three siblings having the same genetic lesion but different clinical presentations

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Background: **This article summarizes the varying clinical manifestations of three siblings with familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) caused by the same genetic lesion.**

Methods: **The medical records of three siblings with FHHNC (one girl and two boys, aged 6 to 12 years) were reviewed and the clinical manifestations and treatment of their disease were described.**

Results: **Despite varying phenotypes, each sibling had the same genetic lesion—a novel homozygous mutation in *CLDN16* (c.211A>G, M71V).**

Conclusion: **Although FHHNC is a rare disorder, this report is significant for the following reasons: (i) it describes a novel *CLDN16* mutation causing FHHNC, adding to the literature of FHHNC-causing *CLDN16* mutations; (ii) it suggests that genes other than *CLDN16* or epigenetic factors are involved in the clinical spectrum of FHHNC; and (iii) it reinforces the variability of disease manifestation and genotype-phenotype correlations.**

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Case report

Introduction

Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) is an autosomal recessive inherited syndrome resulting from mutations in the *CLDN16* and/or *CLDN19* genes that encode claudin-16 and claudin-19, respectively.[1] Although the typical clinical manifestations include renal wasting of magnesium and calcium, leading to nephrocalcinosis and hypomagnesemia, the varied clinical spectra associated with each of these gene mutations are still being actively defined. We report three siblings with FHHNC due to a novel *CLDN16* mutation. Their differing clinical courses highlight the variability of disease manifestations, even among patients with the same genetic mutation. Although rare, FHHNC should be included in the differential diagnosis of any patient with nonspecific constitutional symptoms found to have hypomagnesemia and/or nephrocalcinosis in the setting of renal calcium/magnesium wasting.

Case report

Case 1 (index case)

The index case is a 6-year-old Asian-Indian girl born at term without complications to parents who are second cousins. She was evaluated at 1 year of age for poor oral intake and poor growth (height and weight less than the 3rd percentile for age). She had no history of urinary tract infections (UTIs), polyuria, hematuria, ocular abnormalities or hearing impairment. Laboratory evaluation revealed an elevated blood urea nitrogen (BUN) concentration of 28 mg/dL (reference range: 7-17 mg/dL), and a serum creatinine concentration of 0.5 mg/dL (reference range: 0.1-1.0 mg/dL), giving her a low estimated glomerular filtration rate (eGFR) of 79.2 mL/min per 1.73 m^2 surface area (reference range: >80 mL/min per 1.73 m^2 surface area). She had normal values for serum calcium (9.6 mg/dL; reference range: 8.8-10.6 mg/dL) and magnesium (1.6 mg/dL; reference range: 1.5-2.6 mg/dL). The remainder of the chemistry

profile was unremarkable, with normal values for serum sodium, potassium, chloride, bicarbonate, glucose, and phosphorus concentrations. With a normal urinalysis (pH 6.5, negative glucose and protein; reference range: pH: 4.8-7.8) and serum phosphorus, the possibility of Fanconi syndrome and other proximal renal tubular acidosis was excluded. Furthermore, her urine protein to creatinine ratio was appropriate, eliminating the possibility of Dent's disease. At the time of presentation, her serum 25-hydroxy vitamin D concentration was normal; however, her parathyroid hormone (PTH) levels, measured 2 years after presentation, were elevated (Table).

Four months after her initial visit, the patient was admitted for a timed urine collection which showed an increased urinary calcium excretion. Furthermore, a renal ultrasound performed at two years of age revealed bilateral nephrocalcinosis. The patient then developed recurrent UTIs and was found to have mild to moderate bilateral hydronephrosis at 3 years of age. A left nephrostomy tube was placed at 4 years of age for obstructive nephrolithiasis co-occurring with pyelonephritis and hydronephrosis, which was subsequently removed 2 months later when she was found to have a non-obstructing stone in the upper pole of the left kidney.

Table. Patient parameters at the time of presentation to the University of California, Davis

Parameters	Case 1 (6 y female)	Case 2 (8 y male)	Case 3 (11 y male)
Date	1/5/2005	2/14/2007	2/14/2007
Growth parameters			
Height (percentile)	<3rd	10th	10th
Weight (percentile)	<3rd	3rd	3rd
Date	1/5/2005	6/21/2007	6/21/2007
Serum			
Magnesium (mg/dL, ref: 1.5-2.6)	1.6	1.7	1.6
Calcium (mg/dL, ref: 8.8-10.6)	9.6	9.6	9.5
Blood urea nitrogen (mg/dL, ref: 7-17)	**28**	**52**	**35**
Creatinine (mg/dL, ref: 0.1-1.0)	0.5	1.0	0.9
Parathyroid hormone (pg/mL, ref: 12-88)	**141**	**210**	**97**
Sodium (mEq/L, ref: 136-145)	136	138	138
Potassium (mEq/L, ref: 3.3-5.0)	4.5	**5.3**	4.3
Chloride (mEq/L, ref: 95-110)	101	107	106
Bicarbonate (mEq/L, ref: 22-32)	27	25	25
Glucose (mg/dL, ref: 60-105)	79	92	97
Phosphorus (mg/dL, ref: 3.6-6.8)	6.0	5.8	4.2
Date	5/16/2005	6/30/2007	6/30/2007
Urine (timed collection)			
Calcium (mg/kg/24 h, ref: <4)	**21**	**6.5**	**8.4**
Citric acid/creatinine (mg/g, ref: >180)	517	610	568
eGFR (mL/min/1.73 m², ref: >80)	**79**	**58**	**75**
Date	1/5/2005	2/14/2007	2/14/2007
Urinalysis			
pH (4.8-7.8)	6.5	6	5.5
Specific gravity (1.002-1.030)	1.007	1.015	1.012
Occult blood	negative	negative	**small**
Protein	negative	negative	**30**
Sulfosal	negative	negative	**positive**
Nitrite	negative	negative	negative
Leukocyte esterase	**moderate**	negative	negative

Reference values (where applicable) are given in parentheses. The timing of the studies is described in the text. Values outside the reference range are given in bold.

The patient was treated with Polycitra K at 3 years of age; hydrochlorothiazide was then added at 4 years of age due to persistent hypercalciuria. Amiloride (which increases renal tubular calcium and magnesium reabsorption) and magnesium oxide were subsequently added at 5 years of age. Although early serum magnesium levels were normal, the patient developed mild hypomagnesemia at 4 years of age (magnesium concentration of 1.0 mg/dL). Fortunately, her renal function has remained relatively unchanged over the past 4 years (with current BUN and creatinine concentrations being 26 mg/dL and 0.77 mg/dL, respectively, giving her an eGFR of 76 mL/min per 1.73 m^2 surface area).

Case 2

Patient 2 (a biological brother of the index case) is an 8-year-old boy who was born at term without complications. His early neonatal history was significant for hypocalcemic seizures considered secondary to vitamin D deficiency; however, treatment with calcium and high doses of vitamin D resulted in subsequent vitamin D toxicity and severe hypercalcemia. A renal ultrasound obtained at 6 months of age showed nephrocalcinosis, but no further studies were performed at that time because the nephrocalcinosis was thought to be due to vitamin D toxicity.

However, due to the patient's sister's illness (Case 1), he was evaluated at 5 years of age for a possible inherited metabolic disorder. On physical examination, his height was at the 10th percentile for age and his weight was at the 3rd percentile for age. Laboratory studies revealed hyperkalemia (5.3 mEq/L; reference range: 3.3-5.0 mEq/L), an elevated PTH concentration (210 pg/mL; reference range: 12-88 pg/mL), an elevated BUN concentration (52 mg/dL), and a decreased eGFR (58 mL/min per 1.73 m^2 surface area). The patient's anion gap, his serum calcium, magnesium, sodium, chloride, bicarbonate, creatinine, glucose, and phosphorus concentrations, and his urinalysis were normal (Table). A timed urine collection showed hypercalciuria (Table), and bilateral medullary

nephrocalcinosis was subsequently confirmed on renal ultrasound. The patient has had no UTIs, pyelonephritis, or nephrolithiasis. However, he had occasional nausea and intermittent body aches.

Treatment with polycitra K and hydrochlorothiazide was started at 5 years of age, and magnesium oxide supplementation for mild hypomagnesemia was added at 6 years of age. The patient's renal function remained impaired but stable; his most recent BUN and creatinine concentrations (at 8 years of age) were 34 mg/dL and 1.02 mg/dL, respectively, giving him an eGFR of 64 mL/min per 1.73 m² surface area.

Case 3

Patient 3 is an 11.5-year-old boy (another biological brother of the index case) who was monitored for renal disease given his siblings' medical histories (Cases 1 and 2). At 8 years of age, he was found to have mild bilateral nephrocalcinosis; at that time, his height was approximately the 10th percentile for age and his weight was approximately the 3rd percentile for age. Clinically, the patient reported infrequent enuresis and occasional polyuria. Laboratory studies revealed an elevated PTH concentration (97 pg/mL), an elevated BUN level (35 mg/dL), and a decreased eGFR (75 mL/min per 1.73 m² surface area). Values for serum calcium, magnesium, sodium, potassium, chloride, bicarbonate, glucose, and phosphorus concentrations were normal (Table). A timed urine collection showed hypercalciuria (Table), and his urinalysis was significant for proteinuria. Although initial magnesium levels were normal, he has had persistent hypomagnesemia since 9 years of age (magnesium concentration: 0.8 mg/dL).

At 9 years of age, the patient began to have infrequent headaches and intermittent, vague joint pains; however, he did not have UTIs, pyelonephritis, or nephrolithiasis. At 11 years of age, he was also diagnosed with bilateral myopia (left & right eyes: 20/60). His renal function was also impaired, but remained stable; his most recent BUN and creatinine levels were 26 mg/dL and 0.90 mg/dL, respectively, giving him an eGFR of 82 mL/min per 1.73 m² surface area.

Treatment with polycitra K and hydrochlorothiazide was started at 9 years of age; amiloride was added soon thereafter for persistant hypercalciuria. Because of persistent hypomagnesemia, magnesium oxide was added at 10 years of age.

An analysis of the *CLDN16* gene in all three patients (performed at the Center for Nephrology and Metabolic Disorders, Laboratory for Molecular Diagnostics, Weisswasser, Germany) revealed a novel homozygous mutation in the *CLDN16* gene (c.211A>G, M71V; GenBank accession number NT_005612.16).

Discussion

FHHNC (OMIM 248250) is a rare autosomal recessive disorder caused by mutations in the *CLDN16* and *CLDN19* genes. Both genes encode proteins that are members of the Claudin family, transmembrane proteins found at tight junctions that form paracellular pores and determine ion selectivity of paracellular permeability.[1] *CLDN16*, also called paracellin-1, encodes claudin-16, a protein of 305 amino acids with 4 transmembrane domains critical for the reabsorption of magnesium and calcium from the thick ascending limb of the loop of Henle.[2] Alternatively, *CLDN19* encodes claudin-19, a 224 amino acid protein with 4 transmembrane domains found in the kidney and retina,[3] and patients with an FHHNC-like phenotype due to *CLDN19* mutations have more ocular manifestations than those with *CLDN16* mutations.[3] Claudin-16 and claudin-19 interact to form a cation-selective tight junction complex, and mutated forms of either protein result in a lack of synergistic effects on ion selectivity.[4]

FHHNC manifests clinically with urinary losses of both magnesium and calcium, causing nephrocalcinosis and hypomagnesemia. Affected individuals typically present in childhood with polyuria and recurrent UTIs and are at high risk of developing renal failure.[5,6] Hypocalcemic/hypomagnesemic seizures and ocular abnormalities (macular colobomata, significant myopia, horizontal nystagmus) can also occur.[5,6] Additional symptomatology may include abdominal pain, failure to thrive, vomiting, nephrolithiasis, and rickets.[5,6]

Importantly, this report demonstrates the phenotypic variability in individuals affected with FHHNC—even among related individuals with identical *CLDN16* mutations. Furthermore, although FHHNC is a rare disorder, affected individuals are at high risk for progressive renal failure, with approximately one-third of affected individuals developing end-stage renal disease (ESRD) during adolescence.[7] Thus, given the morbid nature of the condition, pediatricians should maintain a high index of suspicion for FHHNC when a child presents with urinary or constitutional symptoms, hypomagnasemia, and/or nephrocalcinosis, particularly since a diagnostic delay can lead to permanent neurological damage such as psychomotor retardation or seizure disorders.[8]

Treatment for FHHNC typically includes magnesium supplementation. Although not needed in our patients, vitamin D and calcium supplementation are also sometimes required. Thiazide diuretics and potassium/magnesium sparing diuretics (such as amiloride) are helpful in reducing hypercalciuria and calcium/magnesium requirements; however, they have not been shown to be effective in slowing progression of renal disease. Unfortunately, even with this

treatment, the median age at time of ESRD in patients with FHHNC is 14.5 years with a wide range in rate of progression (5.5-37.5 years).[7]

The biological determinant for the progressive tubulointerstitial nephropathy that develops in FHHNC remains unknown. In addition, although all individuals with FHHNC are affected by medullary nephrocalcinosis, their degree of renal insufficiency varies. However, after renal transplantation, renal magnesium and calcium regulation normalize, suggesting that FHHNC patients are ideal candidates for renal transplants.[6] Furthermore, inhibitors of clathrin-mediated endocytosis have been shown to prevent redistribution of renal epithelial cells transfected with mutant *CLDN16* to lysozomes, offering a potential novel therapeutic strategy for FHHNC patients with particular *CLDN16* mutations in the future.[9]

Many different mutations of the *CLDN16* gene are associated with FHHNC,[7,10] and it is not unexpected that each mutation would lead to a different phenotype. In this report, however, we describe three individuals with the same *CLDN16* mutation (c.211A>G; M71V) but different phenotypes and clinical courses. Furthermore, mutations affecting codon 71 of *CLDN16* have been shown to disrupt cell surface expression of the protein and cause complete loss of claudin-16 function.[10] Thus, our finding of different clinical manifestations of FHHNC among those with the same codon 71 mutation in *CLDN16* is interesting in that it not only demonstrates that a specific genotype can have different phenotypic expressions, but suggests that other as yet unidentified genes and/or environmental factors may have a modifying influence on the clinical manifestations of FHHNC.

In conclusion, we describe the varying clinical manifestations of three siblings with FHHNC caused by the same mutation in the *CLDN16* gene. Although FHHNC is a rare disorder, this report is significant for the following reasons: (i) it describes a novel *CLDN16* mutation causing FHHNC, adding to the literature of FHHNC-causing *CLDN16* mutations; (ii) it suggests that genes other than *CLDN16* or epigenetic factors are involved in the clinical spectrum of FHHNC; and (iii) it reinforces the variability of disease manifestation and genotype-phenotype correlations.

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