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BRIEF REPORT

Paracellin-1 gene mutation with multiple congenital abnormalities

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Abstract Familial hypomagnesemia with hypercalciuria and nephrocalcinosis is an autosomal recessive renal tubular disorder characterized by renal magnesium wasting, hypercalciuria, advanced nephrocalcinosis and progressive renal failure. Mutations in the paracellin-1 (*CLDN16*) gene have been defined as the underlying genetic defect. The tubular disorders and progression in renal failure are usually resistant to magnesium substitution and hydrochlorothiazide therapy, but hypomagnesemia may improve with advanced renal insufficiency. We present a patient with a homozygous truncating *CLDN16* gene mutation (W237X) who had early onset of renal insufficiency despite early diagnosis at 2 months. He also had additional abnormalities including horseshoe kidney, neonatal teeth, atypical face, cardiac abnormalities including coarctation of the aorta associated with atrial and ventricular septal defects, umbilical hernia and hypertrichosis. To the best of our knowledge, this is the youngest case diagnosed as familial hypomagnesemia with hypercalciuria and nephrocalcinosis and the first case having such additional congenital abnormalities independent of the disease itself.

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Introduction

Paracellin-1 (claudin-16) is a renal tight junction protein encoded by the *CLDN16* gene [1]. It is a key player in magnesium and calcium reabsorption in the thick ascending limb of Henle's loop [2]. Mutations in *CLDN16* gene lead to familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) [2–4], a rare autosomal recessive tubular disorder that frequently progresses to renal failure [3, 5]. The youngest age at diagnosis has been reported as 0.5 years and the most well-known extrarenal findings in FHHNC are ocular abnormalities and hearing defects [5, 6]. Here we report a case with FHHNC diagnosed at the age of 2 months, who had additional renal and extrarenal findings that have not been defined along with FHHNC before.

Case report

The male patient was born with a body weight of 3,200 g at term following a pregnancy complicated with threatened miscarriage, placental abnormality and thrombosis in one of the umbilical arteries that required salicylate use. His parents were third-degree cousins, and he had two asymptomatic siblings. His mother and paternal uncle had urolithiasis, and three siblings of his father died in the neonatal period. He was admitted to a local hospital with failure to thrive, constipation, atypical appearance of face and cyanosis while crying. Echocardiography revealed aortic coarctation along with atrial and ventricular septal defects. Upon a suspicious

urinary tract infection, abdominal ultrasonography was performed and revealed horseshoe kidney, bilateral medullary nephrocalcinosis and hydronephrosis in the left kidney. He was referred to our clinic for further evaluation when he was 40 days old. His physical examination revealed a body weight of 3,350 g (P3-10), height of 50 cm (P3) and head circumference of 36.5 cm (P10-25) with low-set ears, low-set neck hairline, neonatal teeth, murmur at third and fourth intercostal spaces and apex, umbilical hernia, hypertrichosis at sacral area and lower extremities. Arteriofemoral pulses of the patient were weak in comparison to radial pulses. Blood-pressure measurements at the left and right upper extremities were 125/61 and 112/54 mmHg and at the left and right lower extremities were 98/62 and 93/57 mmHg with a Doppler device. Laboratory findings at admission were blood pH 7.41, pCO_2 37.2 mmHg, HCO_3^- 24 mmol/L, BUN 15.1 mg/dL, serum creatinine (SCr) 0.6 mg/dL, uric acid 3.5 mg/dL, sodium 138 mmol/L, potassium 4.4 mmol/L, magnesium 0.49 (normal 0.7–1.05) mmol/L, calcium 9.8 mg/dL, phosphorus 5.3 mg/dL, alkaline phosphatase 654 U/L, parathyroid hormone 196 (normal 12–72) ng/mL, 25-hydroxy-vitamin D3 28 ng/mL (7.6–75). Urinary calcium excretion was 29 mg kg^{-1} day^{-1} (normal<4), urinary fractional magnesium excretion was 9 and 14% (normal<5%), urinary citrate excretion was 23.6 mg kg^{-1} day^{-1} (normal>2) and urinary urate excretion was 846.5 mg 1.73 m^{-2} day^{-1} (normal>815). Proteinuria was 16 mg/m^2/h and urinary protein electrophoresis revealed albumin dominance. Vesicoureteral reflux was not observed in voiding cystoureterography. Ophthalmologic examination was normal. Bone age was compatible with his age when he was 2 months old. Serum magnesium and urinary calcium/creatinine levels of his parents were in normal ranges. Cytogenetic examination revealed a male karyotype with 46 chromosomes, and blood samples were sent to a center for genetic analysis of the *CLDN16* gene. During his hospitalization, he had cyanosis with bradycardia and tonic convulsions, and electroencephalography showed frontal epileptic foci.

He was prescribed phenobarbital along with hydrochlorothiazide, magnesium citrate, digoxin and spironolactone. When he was hospitalized again for balloon angioplasty after a follow-up period of 2.5 months as an outpatient, laboratory findings were BUN 38.5 mg/dL, SCr 1.03 mg/dL, uric acid 11.9 mg/dL, sodium 134 mmol/L, potassium 3.5 mmol/L, magnesium 0.76 mmol/L, calcium 10.4 mg/dL, phosphorus 6.0 mg/dL and alkaline phosphatase 720 U/L. Unfortunately, 4 days after balloon angioplasty he had cardiac arrest when he was 5 months-old. After his death, genetic analysis revealed a homozygous *CLDN16* mutation (W237X), which resulted in truncation of the protein. In addition, both of the parents were carrying the same mutation in a heterozygous state.

Discussion

FHHNC was first described in 1972 by Michelis et al. [7]. It is a hereditary autosomal recessive disorder related to loss-of-function mutations in paracellin-1 (claudin-16) gene [1, 8, 9]. It is strongly expressed both in the medullary and cortical segments of the loop of Henle in human and rodent kidneys and impaired expression is associated with severe renal calcium and magnesium loss without loss of other electrolytes [10].

Patients with FHHNC usually present during early childhood with recurrent urinary tract infections, polyuria/polydipsia, isosthenuria, renal stones in addition to vomiting, abdominal pain, tetanic episodes or generalized seizures. The youngest age at diagnosis was 0.5 years among the patients reported in the literature [4–6, 10]. Our patient was clinically diagnosed upon nephrocalcinosis that was demonstrated coincidentally in the ultrasonographic examination performed upon urinary tract infection when he was 2 months old. Nephrocalcinosis refers to the diffuse deposition of calcium in the kidney due to increased urinary calcium, oxalate, urate and low amounts of crystal-formation inhibitors like magnesium and citrate [11]. These parameters in the urine, blood gases and serum calcium and phosphate levels must be evaluated in nephrocalcinosis. In addition, plasma magnesium level seems to be an important parameter in evaluation of disorders associated with nephrocalcinosis when FHHNC is considered in the differential diagnosis.

Besides hypomagnesemia, biochemical abnormalities including hypermagnesiuria, hypercalciuria, impaired GFR, and sometimes hypocitraturia and hyperuricemia may occur in FHHNC. Some patients tend to have increased parathyroid hormone (PTH) levels preceding the impairment of GFR [10]. In our patient, laboratory findings demonstrated hypomagnesemia, hypermagnesiuria, hypercalciuria, and hyperuricemia but no hypocitraturia. PTH increased before the rise in serum creatinine levels.

Usually, treatment with magnesium salts, thiazides and potassium citrate are not beneficial in terms of correction in hypomagnesemia and hypercalciuria or slowing down the progression of the renal insufficiency in FHHNC [4–6, 12]. However, hypomagnesemia may completely disappear with a decline in GFR due to the reduction in the filtered magnesium load [4, 10]. Likewise, renal function tests were deteriorated and SCr level reached 1.03 mg/dL in a few months despite medication in our patient, and afterwards a normal plasma magnesium value was achieved.

Parental consanguinity is frequent in FHHNC and Blanchard et al. found a tendency toward hypercalciuria or mild hypomagnesemia in family members with heterozygous *CLDN16* mutations. Furthermore, isolated hypercalciuria or renal stones have been discovered in some

families [5, 13]. Therefore heterozygous relatives at risk are recommended to be screened for *CLDN16* gene mutations and renal complications of hypercalciuria and nephrocalcinosis [13]. Serum magnesium and urinary calcium/creatinine levels were in normal ranges in parents of our patient who both had heterozygous mutations. Nevertheless, his mother passed renal stones. In addition, it would be wise to screen the uncle for the mutation.

Up till now, extrarenal disturbances other than ocular and hearing impairment have not been reported in FHHNC. In our patient, ocular examination was normal, but hearing impairment had not been evaluated yet when he died. During his hospitalization, he had seizures and EEG demonstrated epileptiform foci. Additionally, he had horseshoe kidney, neonatal teeth, atypical face, cardiac abnormalities, umbilical hernia and hypertrichosis. These findings were not attributable to FHHNC or to another defined congenital syndrome. The most similar case reported in the literature was a boy with horseshoe kidney, atypical face and cardiac abnormalities who had a karyotype of 46,XY,del(2)(q37) [14]. Our patient had a normal karyotype, and we thought that presence of the aforementioned abnormalities might be incidental rather than a true association. To our knowledge, this is the first FHHNC case with such multiple independent congenital abnormalities in the literature.

In this report, we presented a patient homozygous for *CLDN16* gene mutation who had a relatively earlier onset of renal deficiency despite early diagnosis and treatment with thiazides and magnesium citrate. By means of the patient, we wanted to emphasize recognition of FHHNC in differential diagnosis of patients with hypercalciuria and nephrocalcinosis, especially in countries where marriages between relatives are still common.

References

1. Simon DB, Lu Y, Choate KA, Velazquez H, Al-Sabban E, Praga M, Casari G, Bettinelli A, Colussi G, Rodriguez-Soriano J, McCredie D, Milford D, Sanjad S, Lifton RP (1999) Paracellin-1, a renal tight junction protein required for paracellular Mg2+ resorption. Science 285:103–106
2. Blanchard A, Jeunemaitre X, Coudol P, Dechaux M, Froissart M, May A, Demontis R, Fournier A, Paillard M, Houillier P (2001) Paracellin-1 is critical for magnesium and calcium reabsorption in the human thick ascending limb of Henle. Kidney Int 59:2206–2215
3. Weber S, Schneider L, Peters M, Misselwitz J, Ronnefarth G, Boswald M, Bonzel KE, Seeman T, Sulakova T, Kuwertz-Broking E, Gregoric A, Palcoux JB, Tasic V, Manz F, Scharer K, Seyberth HW, Konrad M (2001) Novel paracellin-1 mutations in 25 families with familial hypomagnesemia with hypercalciuria and nephrocalcinosis. J Am Soc Nephrol 12:1872–1881
4. Konrad M, Weber S (2003) Recent advances in molecular genetics of hereditary magnesium-losing disorders. J Am Soc Nephrol 14:249–260
5. Benigno V, Canonica CS, Bettinelli A, von Vigier RO, Truttmann AC, Bianchetti MG (2000) Hypomagnesaemia-hypercalciuria-nephrocalcinosis: a report of nine cases and a review. Nephrol Dial Transplant 15:605–610
6. Praga M, Vara J, Gonzalez-Parra E, Andres A, Alamo C, Araque A, Ortiz A, Rodicio JL (1995) Familial hypomagnesemia with hypercalciuria and nephrocalcinosis. Kidney Int 47:1419–1425
7. Michelis MF, Drash AL, Linarelli LG, De Rubertis FR, Davis BB (1972) Decreased bicarbonate threshold and renal magnesium wasting in a sibship with distal renal tubular acidosis: evaluation of the pathophysiological role of parathyroid hormone. Metabolism 21:905–920
8. Nicholson JC, Jones CL, Powell HR, Walker RG, McCredie DA (1995) Familial hypomagnesemia-hypercalciuria leading to end-stage renal failure. Pediatr Nephrol 9:74–76
9. Weber S, Hoffmann K, Jeck N, Saar K, Boeswald M, Kuwertz-Broeking E, Meij IIJ, Knoers NV, Cochat P, Sulakova T, Bonzel KE, Soergel M, Manz F, Schaerer K, Seyberth HW, Reis A, Konrad M (2000) Familial hypomagnesemia with hypercalciuria and nephrocalcinosis maps to chromosome 3q27 and is associated with mutations in the PCLN-1 gene. Eur J Hum Genet 8:414–422
10. Kang JH, Choi HJ, Cho HY, Lee JH, Ha IS, Cheong HI, Choi Y (2005) Familial hypomagnesemia with hypercalciuria and nephrocalcinosis associated with *CLDN16* mutations. Pediatr Nephrol 20:1490–1493
11. Alon US (1997) Nephrocalcinosis. Curr Opin Pediatr 9:160–165
12. Tajima T, Nakae J, Fujieda K (2003) Two heterozygous mutations of *CLDN16* in a Japanese patient with FHHNC. Pediatr Nephrol 18:1280–1282
13. Tasic V, Dervisov D, Koceva S, Weber S, Konrad M (2005) Hypomagnesemia with hypercalciuria and nephrocalcinosis: case report and a family study. Pediatr Nephrol 20:1003–1006
14. Wang TH, Johnston K, Hsieh CL, Dennery PA (1994) Terminal deletion of the long arm of chromosome 2 in a premature infant with karyotype: 46,XY,del(2)(q37). Am J Med Genet 49:399–401