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Case Report

A novel homozygous W99G mutation in *CLDN-16* gene causing familial hypomagnesemic hypercalciuric nephrocalcinosis in Turkish siblings

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SUMMARY: Alparslan C, Öncel EP, Akbay S, Alaygut D, Mutlubaş F, Tatlı M, Konrad M, Yavaşcan Ö, Kasap-Demir B. A novel homozygous W99G mutation in *CLDN-16* gene causing familial hypomagnesemic hypercalciuric nephrocalcinosis in Turkish siblings. Turk J Pediatr 2018; 60: 76-80.

Familial hypomagnesemic hypercalciuric nephrocalcinosis (FHHNC) (OMIM: 248250) is characterized by hypomagnesemia, hypercalciuria and nephrocalcinosis. FHHNC inevitably progresses to end-stage renal disease in decades. Mutations in *CLDN-16 and CLDN-19* genes are associated with disrupted magnesium handling in the thick ascending limp of Henle's loop. Patients with mutations in these genes share similar clinical features, and those with *CLDN-19* gene mutations have ocular findings in addition.

A 2-month-old boy, was admitted to our clinic with the complaints of upper respiratory tract infection. He was the first-born child of consanguineous parents. Laboratory findings revealed hypocalcemia and hypomagnesemia. Bilateral medullary nephrocalcinosis was detected on abdominal ultrasound. His ophthalmologic examination was unremarkable. With hypomagnesemia, hypercalciuria and nephrocalcinosis, the patient was considered to have FHHNC. Oral magnessium supplementation was initiated. Four years of follow-up has been completed uneventfully.

When 6-days-old the brother of the case above was admitted with seizure. The patient was resistant to calcium and anticonvulsant drugs and the seizure activity could only be controlled after magnesium infusion. Biochemistry profile revealed hypocalcemia and hypomagnesemia. Urinary calcium extraction was 11 mg/kg/day. Medullary nephrocalcinosis was reported on renal ultrasound. His eye examination, echocardiography, transfontanel ultrasound and electroencephalography were normal. Due to the triad of hypomagnesemia, hypercalciuria and nephrocalcinosis, and the medical history of his elder brother, he was diagnosed with FHHNC. After correction of the electrolyte abnormalities, he was discharged from hospital and is currently being followed-up without any problem.

In this manuscript, we shared our experience about a novel homozygous mutation (W99C) in *CLDN-16* gene causing FHHNC in a couple of Turkish siblings.

Key words: nephrocalcinosis, hypomagnesemia, hypercalciuria, children.

Familial Hypomagnesemic Hypercalciuric Nephrocalcinosis (FHHNC) (OMIM: 248250) is a rare autosomal recessive disease with a triad of hypomagnesemia, hypercalciuria and nephrocalcinosis.[1] In comparison to distal renal tubular acidosis (dRTA), medullary sponge kidney and primary hyperparathyroidism which are also characterized by medullary nephrocalcinosis, FHHNC is a devastating condition leading to end-stage renal disease

(ESRD).[2] Mutations in the *CLDN* genes (*CLDN-16 and CLDN-19*), encoding renal tight junction proteins (claudin-16 and claudin-19) playing key roles in magnesium handling in the thick ascending limb of Henle's loop, result in FHHNC. Both mutations cause a similar renal phenotype and *CLDN19* mutations result in additional ocular involvement.[3,4] Here, we report a novel homozygous mutation in *CLDN-16* gene affecting two Turkish siblings.

Case Reports

Case 1

A 2-month-old boy, was admitted to our clinic with the complaints of upper respiratory tract infection. He was the first-born child of consanguineous parents (Fig. 1). In his physical examination, body weight was 6.87 kg (P 75-90), height was 60 cm (P 75-90), and the rest of the physical examination was unremarkable.

Laboratory findings revealed hypocalcemia (initial: 6.8 mg/dl and control 7.4 mg/dl) and hypomagnesemia [1.1 mg/dl (N: 1.5-2.4)]. Other parameters were as follows: serum urea 10 mg/dl, serum creatinine 0.4 mg/dl, potassium 4.57 mmol/l, sodium 136 mmol/l, phosphorus 5.9 mg/dl, serum parathyroid hormone 299.5 ng/ml (N: 15-65), 25-OH vitamin D 5.79 ng/ml (N: 20-32); urinary calcium/creatinine 1.17 (N:<0.4), magnesium/creatinine 2.2 (N:0.04-1.9 mmol/mmol), oxalate/creatinine: 134.62 (N: 5.40-444 mg/g), citrate/creatinine: 1.28 (N: >0.51 g/g), cystine/creatinine: 315.3 (N: 7-54 umol/mmol). Also, bilateral medullary nephrocalcinosis was detected on abdominal ultrasound. His ophthalmologic examination was unremarkable. With hypomagnesemia, hypercalciuria and nephrocalcinosis, patient was considered to have FHHNC. Therefore, oral magnesium supplementation was initiated but thiazide treatment could not be given due to unavailability in our country. Four years of follow-up has been completed uneventfully. His last serum creatinine level was 0.8 mg/dl (GFR:76 ml/min/1.73 m^2) and he has growing kidneys on ultrasound [Dimensions: right kidney long axis 73 mm (N: 60-80 mm) and parenchymal thickness 7mm; left kidney long axis 76 mm (N: 60-75 mm) and parenchymal thickness 7 mm despite persistent medullary nephrocalcinosis] (Fig. 2).

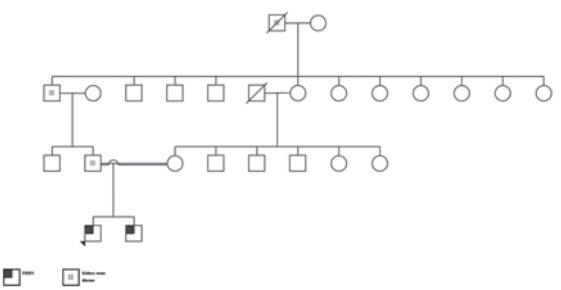

Fig. 1. Pedigree of the family.

Case 2

When 6 days old the sibling of the case mentioned above was admitted with seizure. The patient was resistant to calcium and anticonvulsant drugs and the seizure activity could only be controlled after magnesium infusion. He was born by vaginal delivery with a birth weight of 3900 g to a 24-year-old healthy woman with no history of drug usage. His prenatal history was unremarkable. His weight was 4200 g (P 90-97), length was 56 cm (P 97), and head circumference was 37 cm (P 50-90). His biochemistry profile revealed hypocalcemia (calcium: 6.9 mg/dl) and hypomagnesemia (magnesium: 1.2 mg/dl). Phosphorus level was 8.9 mg/dl, parathormone level was 106 pg/ml (N: 15-65), 25-hydroxy vitamin D level was 5.24 ng/ml. Other parameters were normal. Calcium, magnesium and vitamin D were replaced according to repeated laboratory results. Meanwhile, calcium extraction was 11 mg/kg/day. Medullary nephrocalcinosis was reported on renal ultrasound. His eye examination, echocardiography, transfontanel ultrasound and electroencephalography were normal. Due to the triad of hypomagnesemia, hypercalciuria and nephrocalcinosis, and the medical history of his elder brother, he was diagnosed with FHHNC. After correction of the electrolyte abnormalities, he was discharged from hospital and is currently being followed-up without any problem.

The genetic analysis revealed a new homozygous mutation (W99G) in *CLDN16* in both siblings and both parents were for heterozygous for the mutation.

Written informed consent was obtained from family about genetic analysis and publication of the manuscript.

Discussion

FHHNC is an autosomal recessive disorder

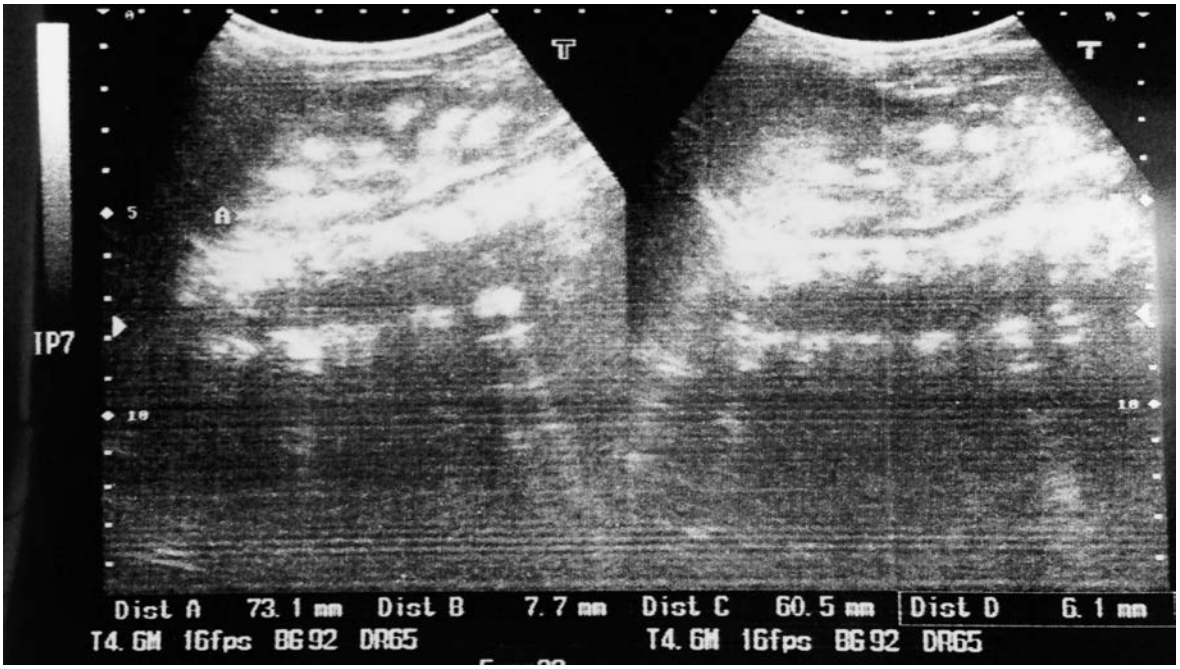

Fig. 2. Bilaterally medullary nephrocalcinosis in kidney ultrasonography.

caused by loss-of-function mutations of genes encoding for tight junction proteins named claudins (16 & 19) responsible for magnesium and calcium reabsorption in the thick ascending loop of Henle. As a result, hypermagnesiuria, hypomagnesemia, hypercalciuria, hypocalcemia and nephrocalcinosis are seen in patients who have mutations in *CLDN16* and *CLDN19*. As Claudin-19 is also expressed in the retina, mutations in CLDN19 cause additional ocular problems.[4] We reported two siblings with a new defined homozygous mutation in *CLDN16*.

Both of our patients had hypermagnesiuria, hypocalcemia and increased parathyroid hormone levels preceding the impairment of GFR in addition to the diagnostic triad and had no ocular problems. These findings suggest a mutation in *CLDN16* gene, which is located in chromosome 3q and consisting of 5 exons.[5] Various mutations have been identified up till now. The designation of the mutations is: CLDN16 c.295g>t; p.W99G indicating the changes at the nucleotide (c) and protein (p) level (Fig. 3). The amino acid residue is located in the first extracellular loop and the aminoacid exchange changes tryptophane (polar) to a nonpolar aminoacid residue (glycine). These mutations are not assigned in public databases (exome variant server, 100 genomes). In silico prediction judges this variant as pathogenic (POLYPHEN2 score 0.999).

Patients with FHHNC usually present with recurrent urinary tract infection, polyuria and polydipsia. Additionally, recurrent renal stones, rickets, hematuria, muscular tetany, seizures, failure to thrive, vomiting and abdominal pain may be detected.[3-5] Our first patient was diagnosed upon incidentally detected hypocalcemia. In the second one, recurrent seizures resistant to treatment due to hypocalcemia and hypomagnesemia in the early neonatal period was the presenting sign. In the neonatal period, maternal diabetes, intrauterine growth restriction, maternal magnesium deficiency, malabsorption syndrome, inherited disorders related to renal wasting magnesiuria secondary to furosemide or aminoglycosides and neonatal hypoparathyroidism can cause hypomagnesemia.[6] However, neonatal hypomagnesemic convulsion due to FHHNC has not been defined yet. The typical clinical manifestations of hypomagnesemia with secondary hypocalcemia in infants are recurrent generalized convulsions, tetany or irritability. These symptoms are refractory to calcium supplementation and respond only to intravenous or oral magnesium therapy.[7] We managed our patient initially with intravenous magnesium (2.5 to 5.0 mg/kg of elemental magnesium) and then maintained with oral

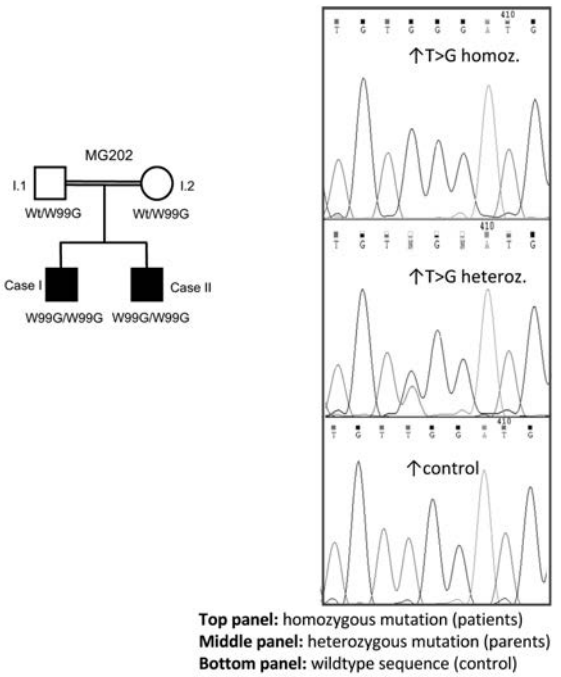

Fig. 3. Family pedigree and the designation of the mutations is: CLDN16 c.295g>t; p.W99G indicating the changes at the nucleotide (c) and protein (p) level.

magnesium oxide supplementation (25 mg/kg/day) without any problem. Like neonatal hypomagnesemia, neonatal hypocalcemia is caused by prematurity, fetal growth retardation, infants of diabetic mother, birth aspyhxia, hypoparathyroidisim, syndromes (e.g. DiGeorge syndrome, CATCH), hypomagnesemia, vitamin D deficiency.[8,9] Although, neonatal hypocalcemic seizures due to maternal vitamin D deficiency is not well studied in western societies, symptomatic hypocalcemia without evidence of rickets may present in the early neonatal period.[9,10] Hypocalcemia and vitamin D deficiency may have additive effect in seizures in our patient. After all, we speculate that renal calcium and magnesium wasting is the major pathway for both symptomatic hypocalcemia and hypomagnesemia in our patients.

Medullary nephrocalcinosis was a noteworthy finding in our patients. It may be associated with primary hyperparathyroidism, distal renal tubular acidosis, medullary sponge kidney, hypervitaminosis D, Williams-Beuren syndrome, primary hyperoxaluria, Dent's disease and FHHNC. Among mentioned pathologies, hyperoxaluria, Dent's disease and FHHNC are associated with ESRD.[2,3] Thus, FHHNC should be considered and other laboratory parameters should be detected while evaluating patients with medullary nephrocalcinosis even in the neonatal period.

In the literature, diagnosis of FHHNC spans a wide range of age. It seems to be clustered at 2–3 years of age.[2,4,5,11-16] This relatively late age of diagnosis indicates that clinicians are not aware of this entity. Strikingly, our patients were diagnosed in an early period of their lives. As we were familiar with FHHNC because of our previous patients[3,14], the elder brother was diagnosed when he was 2-months-old and the younger was diagnosed in the postnatal day 6 because of the medical history of his brother. To the best of our knowledge, our second case was the youngest patient diagnosed with FHHNC in the literature.

There is still a debate on whether early diagnosis could alter the disease progression.[13,15,16] It is known that palliative treatment e.g. magnesium and calcium supplementation, does not have the ability to slow down the disease progression and the definitive treatment of the FHHNC is kidney transplantation.[2,4,5,11-16] We are planning a close follow-up for our patients.

In conclusion, FHHNC is a rare and devastating disease progressing to ESRD. Practitioners should keep FHHNC in mind in the differential diagnosis of patients with hypocalcemia, hypomagnesemia and medullary nephrocalcinosis and even in the neonates with hypomagnesemic and hypocalcemic convulsions, especially in countries like ours, where consanguineous marriages are common. In addition, we report the youngest case with FHHNC in this paper.

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