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BRIEF REPORT

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Two heterozygous mutations of *CLDN16* in a Japanese patient with FHHNC

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Abstract Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC, MIN 248250) is a rare autosomal recessive tubular disorder that eventually progresses to renal failure. However, the progression to end-stage renal failure can vary from patient to patient. A primary defect is related to impaired tubular resorption of magnesium and calcium in the thick ascending limb of Henle's loop. Recently, paracellin-1 was identified as a renal tight junction protein predominantly expressed in TAL. Mutations of its gene *(CLDN16*) have been shown to cause FHHNC. We describe a sporadic Japanese case of FHHNC. The male patient showed hematuria, hypercalciuria, and nephrocalcinosis at 5 years of age. Hypomagnesemia was also noticed at this time. As renal function gradually deteriorated, further evaluation was performed at 14 years of age and a diagnosis of FHHNC was made. Despite several medications (magnesium supplementation, citrate, and hydrochlorothiazide), he eventually progressed to renal insufficiency at 19 years of age. Analysis of the *CLDN16* gene demonstrated two heterozygous mutations (R149Q and R216C). Mutations of the same amino acids have already been described in FHHNC and thus these mutations might be the cause of the disease in our patient. Hence, we confirm the genetic impairment of the *CLDN16* gene in a Japanese patient with FHHNC.

Keywords Familial hypomagnesemia with hypercalciuria and nephrocalcinosis · Paracellin-1 · *CLDN16* · Renal insufficiency

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Introduction

Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC, MIN 248250) is a rare autosomal recessive disorder [1]. FHHNC is characterized by hypomagnesemia, hypercalciuria, advanced nephrocalcinosis, and progressive renal failure [1, 2, 3, 4, 5, 6]. A primary defect of FHHNC is the impairment of the resorption of magnesium in the medullary thick ascending limb of the loop of Henle (TAL) [5]. The clinical course of FHHNC is heterogeneous and the progression to end-stage renal failure is quite variable [6, 7, 8, 9, 10]. The reason for this clinical variability has not yet been clarified. However, it is generally considered that treatments such as magnesium supplementation, citrate, and thiazide diuretics are not effective in preventing the progression of the disease [6, 8, 10].

Recently, paracellin-1 (PCLN-1) was identified as a renal tight junction protein predominantly expressed in TAL [11]. Mutations of its gene *(CLDN16*) have been shown to be the cause of FHHNC [10, 11, 12]. Most mutations are found in the first and second extracellular loop [5, 10, 11, 12].

In this study we report the clinical course of one sporadic Japanese patient with FHHNC. Analysis of the *CLDN16* gene identified two heterozygous missense mutations (R149Q and R216C).

Case report

The male patient was born without complications at term after a normal pregnancy. His maternal uncle was reported to have renal stones, but the exact medical history was not available. His parents are not consanguineous and did not show any symptoms of renal disease. He was admitted to the local hospital because of hematuria at 5 years of age. Laboratory investigations showed hypocalcemia and hypercalciuria. Abdominal computed tomography demonstrated bilateral nephrocalcinosis. The etiology of the hypercalciuria and nephrocalcinosis remained unexplained and no medical treatment was given at this time. Urological management for renal stones was required twice. Since renal function gradually deteriorated, he was referred to our hospital and admitted for further

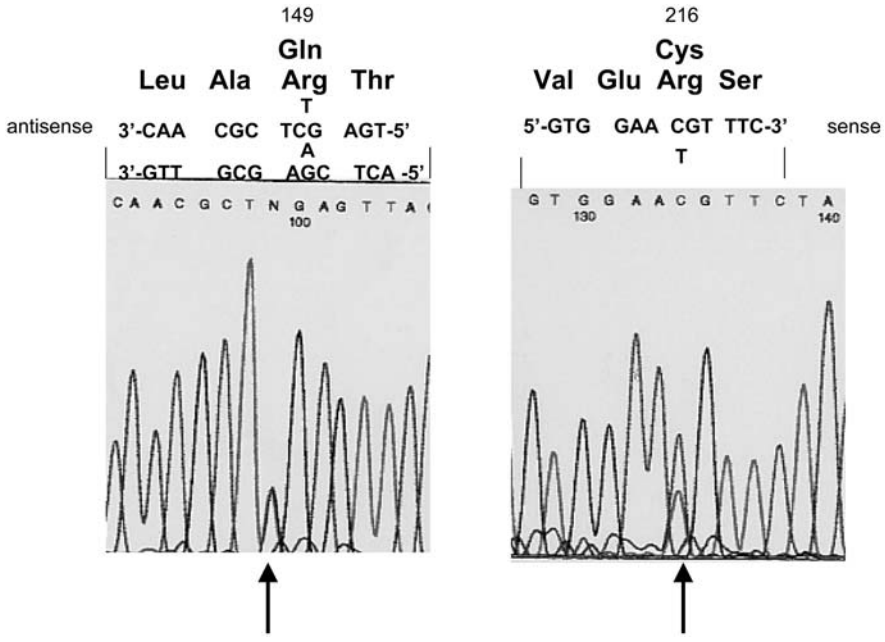

Fig. 1 Mutations of *CLDN16*. DNA sequences are shown. *Arrows* indicate mutation sites. CGA (Arg) to CAA (Gln) was identified at position 149 (DNA sequence shows the antisense strand). A second heterozygous mutation was at position 216 [CGT (Arg) to TGT]

evaluation at 14 years of age. Blood pressure values were normal. Eye abnormalities were not found. Serum levels of creatinine and urea nitrogen were elevated (118 μmol/l and 13.5 mmol/l, respectively, normal range, serum creatinine 27–62 μmol/l, urea nitrogen 1.8–6.4 mmol/l). Serum magnesium and calcium levels were low (0.32 mmol/l and 1.95 mmol/l, normal range, magnesium 0.65–1.0 mmol/l, calcium 2.1–2.7 mmol/l). Serum levels of sodium, potassium, phosphorus, and alkaline phosphatase were within the normal range. Proteinuria (800–1,400 mg/day) was found. Fasting urinary calcium to creatinine ratios (mmol/mmol) (0.52–0.72) were above the normal range (0.14–0.42). The urinary fractional magnesium excretion (9%–12.8%) was elevated (normal range 1.6%–8.1%). Treatment with hydrochlorothiazide (1.8 mg/kg per day), citric acid (0.08 g/kg per day), and magnesium citrate (0.9 mmol/kg per day) was begun. Despite these medical treatments, he progressed to end-stage renal failure and required dialysis at 19 years of age.

DNA amplification and sequence analysis

Informed consent for participation in the study was obtained from the patient. Genomic DNA was extracted from peripheral leukocytes and each exon of the *CLDN16* gene was amplified by polymerase chain reaction (PCR) using primers previously described [12]. The PCR conditions consisted of 9 min at 94°C followed by 30 cycles of 30 s at 94°C, 30 s at 52°C, and 30 s at 72°C in a Perkin-Elmer Gene Amp PCR System 2400 thermal cycler (PE Applied Biosystems, Foster City, Calif., USA). After amplification, the PCR products were purified from low-melting agarose gel, and the purified products were sequenced directly with an ABI PRISM Dye Terminator Cycle Sequencing Kit and an ABI 373A automated fluorescent sequencer (PE Applied Biosystems).

Results

We identified a heterozygous point mutation in exon 2 of the *CLDN16* gene at position 149 [CGA (Arg) to CAA (Gln)] (Fig. 1). A second heterozygous mutation was found at position 216 [CGT (Arg) to TGT (Cys)] (Fig. 1) in exon 3. These two mutations were not found in 50 normal Japanese subjects. DNA was not available from the patient's parents.

Discussion

Our patient showed typical clinical and biochemical abnormalities of FHHNC. After 14 years follow-up, he reached end-stage renal failure despite treatment. Some patients exhibit a rapid decline of renal function [6, 7, 8, 10]. In our patient progression to end-stage renal failure took 14 years despite no medical treatment from 5 to 14 years of age. It is possible that early initiation of medical treatment (magnesium, citrate, and hydrochlorothiazide) might have further delayed the progression of renal disease in our patient. In particular, hydrochlorothiazide reduces urinary calcium excretion and is used for hypercalciuria. However, Wolf et al. [8] reported that although hydrochlorothiazide reduced the calcium/creatinine ratio in their patients, nephrocalcinosis and the progression to renal insufficiency could not be prevented. They suggested that an unknown modifier gene might influence the clinical course.

Praga et al. [6] reported that six of eight patients required chronic dialysis after 1–7 years. Weber et al. [10] reported a median age for end-stage renal failure of 14.5 years (range 5.5–37.5 years). A recent study of seven Arab patients by Kari et al. [9] demonstrated a slow progression to renal failure. They suggest that the

relatively slow progression to renal failure compared with studies from Europe may be due to ethnic differences. Of course, we cannot draw any conclusions on racial differences of European, Arab, and Japanese patients with FHHNC. Analysis of more Japanese patients is needed to answer this question.

Sequence analysis identified two heterozygous mutations (R149Q and R216C). To date several mutations of the *CLDN16* gene have been reported in FHHNC patients. These include 21 missense, 2 frameshift, 2 nonsense, and 3 splicing mutations [5, 10, 11, 12]. In our study, one mutation was R149C. This arginine in the first extracellular loop is highly conserved among claudin family members [11]. In FHHNC, mutations of the same amino acid have been described (R149X and R149L in a German patient) [10, 11]. The other mutation in our study was R216C. The substitution of threonine for this arginine (R216T) has also been reported in one Algerian family with FHHNC [10]. These mutation findings might indicate a mutation cluster but more patients should be analyzed.

The patient′s maternal uncle was reported to have renal stones. A recent large study by Weber et al. [10] demonstrated that heterozygous mutations of *CLDN16* cause hypercalciuria and nephrolithiasis in family members not affected by FHHNC. *CLDN16* is a candidate gene for idiopathic hypercalciuria. In our case, the maternal uncle might have a heterozygous mutation of *CLDN16*.

In conclusion, we report a sporadic Japanese patient with FHHNC and confirm two mutations of the *CLDN16* gene. Further study of Japanese patients with FHHNC is needed to establish ethnic differences of clinical course and the distribution of mutations of the *CLDN16* gene.

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