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A novel mutation in *CLDN16* results in rare familial hypomagnesaemia with hypercalciuria and nephrocalcinosis in a Chinese family

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ABSTRACT

Background: Familial hypomagnesaemia with hypercalciuria and nephrocalcinosis (FHHNC) is a rare autosomal recessively inherited disease characterized by excessive wasting of renal tubular magnesium and calcium. FHHNC is associated with various mutations in *CLDN16* and *CLDN19*.
Cases: Two children from a consanguineous family of Chinese Han origin demonstrated manifestations of rickets, polyuria, polydipsia, hematuria and failure to thrive. Hypomagnesaemia (0.49–0.50 mmol/L), hypercalciuria or a trend to hypercalciuria (24 hour urine calcium: 3.8–5.1 mg/kg/day), and secondary hyperparathyroidism (serum PTH level: 94.7–200 pg/mL) were revealed upon laboratory examination. Using targeted next-generation sequencing and subsequent confirmation by Sanger sequencing, a novel homozygous mutation was identified in the *CLDN16* gene of both FHHNC patients. This specific mutation, a 16 bp deletion followed by a 23 bp insertion in exon 3, led to the generation of a premature termination codon. The parents and an unaffected sister were all heterozygous carriers of this mutation.
Conclusions: We detected a novel mutation in *CLDN16* for the first time. The clinical and genetic findings from this study will help to expand the understanding of this rare disease, FHHNC.

1. Introduction

Familial hypomagnesaemia with hypercalciuria and nephrocalcinosis (FHHNC) is a rare autosomal recessively inherited disease characterized by excessive wasting of renal tubular magnesium and calcium, which ultimately leads to progressive renal failure during childhood or adolescence [1–2]. Common symptoms of FHHNC include nephrolithiasis, recurrent urinary tract infections, polyuria, polydipsia, hematuria, muscular tetany, rickets and failure to thrive. Common laboratory findings include hypomagnesaemia, hypercalciuria, elevated serum intact parathyroid hormone (PTH) levels, incomplete distal renal tubular acidosis (dRTA), hypocitraturia, hyperuricemia, and an impaired glomerular filtration rate [3–6].

FHHNC is associated with mutations in *CLDN16* (OMIM 248250) and *CLDN19* (OMIM 248190), which encode claudin-16 (initially named paracellin-1) and claudin-19, respectively [7–8]. Claudins are important transmembrane proteins that span the plasma membrane four times, and possess a carboxy- and amino-terminal in the cytosol. Claudin protein domains have been shown to have different functions. The first extracellular loop affects paracellular charge selectivity; the second loop is likely responsible for the interaction between opposing claudins of adjacent cells; and the carboxy-terminal cytosolic tail plays roles in protein stability and trafficking to tight junctions [9–10]. Claudin-16 interacts with claudin-19 to form heteromultimers in the tight junctions of the thick ascending limb of Henle in the kidney where they are involved in paracellular reabsorption of calcium and magnesium, a process driven by lumen-positive transepithelial potential [11]. Therefore, there is no discernible difference in renal phenotypes between patients with mutations in *CLDN16* and *CLDN19*. As *CLDN19* is also highly expressed in human eye tissue, FHHNC associated with *CLDN19* mutations may have differing extra-renal phenotypes, such as severe ocular abnormalities [8].

At present, a total of 57 mutations in *CLDN16* and approximately 16 mutations in *CLDN19* have been identified (http://www.hgmd.cf.ac.uk). The majority of previously studied patients with FHHNC came from European, Middle Eastern and North African countries, and only a few had been reported in USA, East Asia or South Asia [3]. Currently, only one patient with a *CLDN19* mutation and two patients with *CLDN16* mutations were reported in two Chinese families [12–13]. Therefore, our study aimed to detect and confirm the pathogenic mutations of *CLDN16* or *CLDN19* in two children with FHHNC in one Chinese family, and to investigate the phenotypes of the two patients in detail.

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2. Materials and methods

2.1. Subjects

Five members from a consanguineous family of Chinese Han origin were included in the study. The pedigree of the family is shown in Fig. 1. Two children presented with rickets, bilateral medullary nephrocalcinosis and hypomagnesaemia in the Department of Endocrinology, Peking Union Medical College Hospital (PUMCH) in 2015, and were suspected of having FHHNC based on clinical and laboratory findings. 100 unaffected, unrelated, healthy subjects were recruited as part of a normal control group for genetic analyses. This study was approved by the Ethics Committee of PUMCH. Signed informed consent was obtained from all patients before participation in the study.

2.2. Clinical evaluation

Serum levels of magnesium (sMg), calcium (sCa), phosphorus (sP), alkaline phosphatase (ALP, bone formation marker), creatinine (sCr), as well as 24 h urinary concentrations of magnesium (uMg), calcium (uCa) and creatinine (uCr) were measured using an automatic biochemistry analyzer. The arterial blood gas (ABG) was analyzed spectrophotometrically using routine assays at the central laboratory of PUMCH. Serum levels of cross linked C-telopeptide of type I collagen (β-CTX, bone resorption marker), 25-hydroxyvitamin D (25OHD) and PTH were measured by an automated Roche electrochemiluminescence system (Roche Diagnostics, Switzerland). Serum levels of 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] were determined by a ^{125}I radioimmunoassay (DPC, USA). Hypomagnesaemia was defined as a serum Mg level <0.7 mmol/L. Fractional urinary Mg excretion (FEMg%) was calculated based on urinary and serum concentrations of Mg and Cr using the following formula: (uMg × sCr) / (0.7 × sMg × uCr) × 100%. Renal Mg loss was considered excessive when FEMg% value was >4% [14]. Hypercalciuria was defined as a 24 hour urinary calcium level above 4 mg/kg/day. Estimated glomerular filtration rate (eGFR) was calculated using the revised Bedside Schwartz formula [15].

Bone deformities at the wrist, skull and thoracolumbar spine were evaluated by X-ray films. Renal cysts, nephrocalcinosis or calculi were assessed by standard ultrasound scanning of the kidney and urinary tract (SIEMENS, Germany). Growth rate was assessed based on percentile charts for standard height and weight for Chinese children [16].

2.3. Genetic analyses

Genomic DNA was extracted from peripheral white blood cells using the DNA Extraction Kit (QIAamp DNA; Qiagen, Germany). The pathogenic mutation was identified using a targeted next-generation sequencing (NGS) panel (Illumina HiSeq2000 platform, Inc., San Diego, CA, USA). >700 genes related to bone disorders and hypomagnesaemia were included in this panel, including (but not limited to) *CLDN16*, *CLDN19*, *TRPM6*, *EGF*, *ATP6*B1, *ATP6V0A4*, *SLC4A1*, and *VDR*. Sequencing was performed using the Illumina HiSeq2000 platform (110-bp paired-end sequencing) according to the standard protocol. The overall sequencing coverage of the target regions was ≥98.95% for a 200 × depth of coverage in each chromosome. Variant filtering of the data was done assuming autosomal recessive inheritance according to pedigree of this consanguineous family. We included variants of frameshift, nonsense, missense, and acceptor and donor splice site, and variants with minor allele frequency (MAF) < 0.5% in the Single Nucleotide Polymorphism Database (dbSNP build 137), the 1000 Genomes Project, the National Heart, Lung, and Blood Institute (NHLBI), the Exome Sequencing Project Exome Variant Server (EVS),

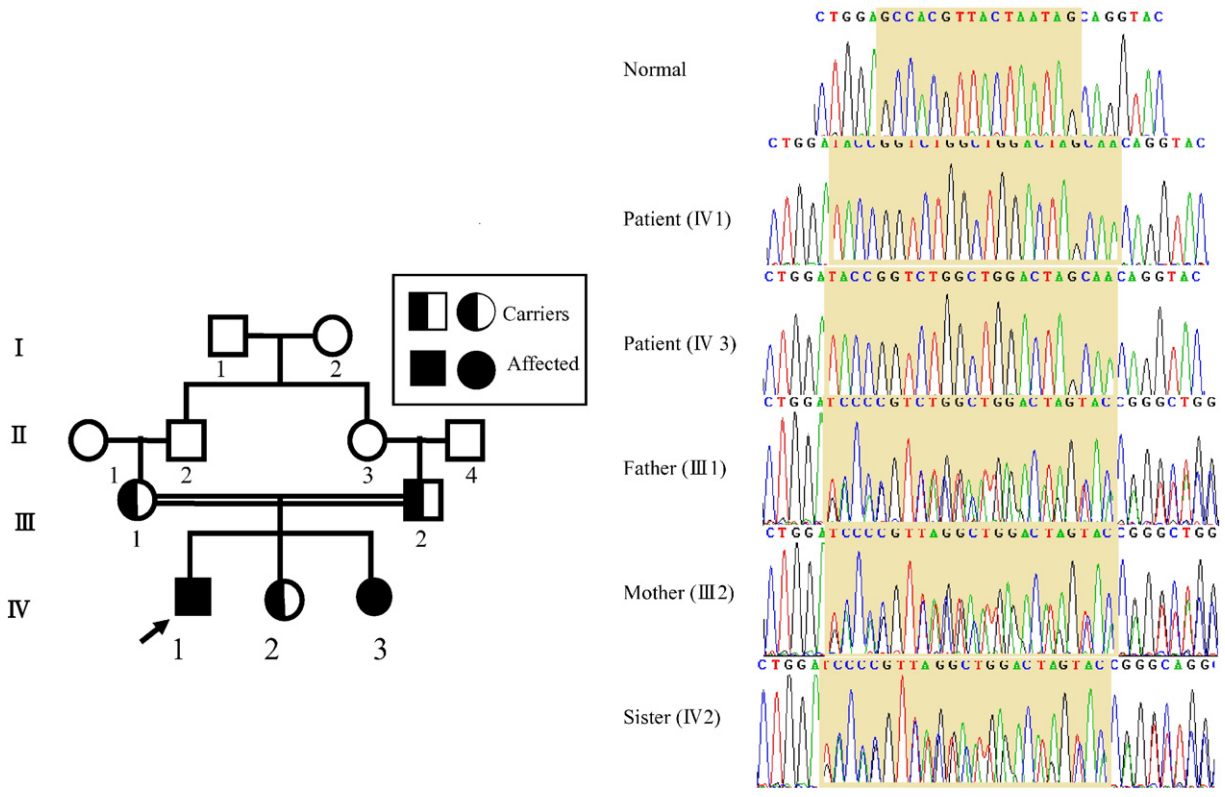

Fig 1. Pedigree of the FHHNC family in this study. The proband was designated with an arrow. Sanger sequencing results of two patients, their parents and sister. In patient IV1 and IV3, a novel homozygous mutation was identified as c.574_589delinsTACCGGTCTGGCTGGACTAGCAA in exon 3 of *CLDN16*. Their parents and sister (IV2) were asymptomatic heterozygous carriers for the mutation.