A Novel Mutation in CLDN16 Gene Causing Familial Hypomagnesemia, Hypercalciuria, Nephrocalcinosis in An Iranian Family

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Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) is a rare autosomal recessive disorder that is characterized by renal magnesium wasting, hypercalciuria and eventually kidney failure which mostly affects children and young aged adults. Mutation of genes of claudin-16 and claudin-19 are involved in the pathogenesis of this disorder, which leads to renal magnesium and calcium wasting.

A 35-year-old man with end-stage kidney disease (ESKD) was referred to our clinic due to bilateral nephrocalcinosis, detected by ultrasonographic study, for further evaluation. Detailed investigations revealed that his siblings had also similar presentations of hypomagnesemia, hypercalciuria, nephrocalcinosis and chronic kidney disease (CKD). Sanger sequencing showed a novel mutation (c.338G > A: p.C113Y) at the second exon of the *CLDN16* gene. The patient underwent kidney transplantation and his siblings received only medical treatment.

In young patients with ESKD and concomitant nephrocalcinosis, especially where there is a family history of CKD/ESKD, genetic evaluation is strongly recommended.

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Case Report

INTRODUCTION

Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) is a rare autosomal recessive disorder characterized by renal magnesium wasting, hypercalciuria and progressive kidney failure, although the absence of hypomagnesemia does not rule out FHHNC.[1,2,5] Fifty percent of these patients will eventually require kidney replacement therapy at the second decade of life.[3,4]

FHHNC is a clinical condition caused by mutations of *CLDN 16*, situated on the long arm of chromosome 3, and *CLDN19*, situated on the short arm of chromosome 1. Proteins that are encoded by these two genes are claudin-16 and claudin-19, which belong to a group of tight junction proteins. Both proteins are expressed in the thick ascending limb of the loop of Henle (TAL).[1,6,7]

Interaction of these two proteins is necessary for their integration into the tight junctions of TAL. This interaction will result in effective paracellular reabsorption of magnesium and calcium ions along the TAL.[7,8,9,10] Therefore, any malfunction in claudin-16 or claudin-19 proteins would potentially lead to renal wasting of magnesium and calcium ions.[11,12] Claudin-19 is also expressed in tight junctions of the retina. Therefore, a mutation in claudin-19 could also cause severe visual disorders.[7,13]

Sixty-nine different mutations of the *CLDN-16* gene have been recognized, which are mostly of

missense or nonsense types and 22 mutations have been described for the *CLDN-19* gene.[14]

In most cases of FHHNC kidney transplantation is the only treatment of choice. [1,15,16,17] Administration of magnesium, calcium, or hydrochlorothiazide may only slow the progression to ESKD.[16] In this study, three FHHNC cases are reported and described in detail.

CASE REPORT

A 35-year-old Kurdish man with ESKD was referred to our nephrology clinic due to bilateral medullary nephrocalcinosis, as reported in ultrasonographic studies (Patient 1). He had been receiving regular hemodialysis for the past two years. A history of frequent nephrolithiasis treated with multiple sessions of extracorporeal shock wave lithotripsy (ESWL) was also noted since his early adulthood. Further investigation demonstrated hypomagnesemia, hypercalciuria, nephrocalcinosis and chronic kidney disease in his brother (Patient 2) and sister (Patient 3) (table1). No visual defect was detected in the patient and his family members. Physical examinations were normal. His parents had a consanguineous marriage.

Whole exome sequencing (WES) was performed. A novel pathogenic variant of a missense mutation (c.338G > A: p.C113Y) in exon 2 of the *CLDN*16 gene was found, meeting the criteria of the American College of Medical Genetics (Figure 1). The results were subsequently confirmed by Sanger sequencing (Figure 2). This new missense mutation of the *CLDN16* gene was also identified in his siblings, although his daughter and his parents were asymptomatic. This was congruent with the results of Sanger sequencing, showing that they are heterozygous for this mutation.

The patient underwent kidney transplantation from a deceased donor. The renal allograft function remained well with no hypomagnesemia or nephrocalcinosis detected within two years of transplantation, until the time of this report. His siblings received medical treatment with high dose magnesium.

DISCUSSION

Here we report a patient with ESKD caused by FHHNC and missense mutation in exon 2 of *CLDN*16 gene, who finally underwent successful kidney transplantation. The same mutation was found in two of his siblings with clinical features of nephrocalcinosis, hypercalciuria and hypomagnesemia and CKD.

Demographic Characteristics and Laboratory Results

Demography	Patient 1	Patient 2	Patient 3
Gender	Male	Male	Female
Date of Diagnosis	2018	2018	2018
Age at Diagnosis	35	24	33
Laboratory Findings at Diagnosis			
Serum Magnesium, mg/dL	Nd	1.6	1.57
Serum Creatinine, mg/dL	8.2	1.3	2.4
eGFR, mL/min/ 1.73m^2	6 (ESKD)	105	40
Serum Calcium, mg/dL	8.6	8.3	8.5
Serum Phosphate, mg/dL	3.4	2	3.2
Serum Potassium, mEq/L	4.2	4	4.4
PTH, pg/mL	341	Nd	147
25 OH Vitamin D Level, ng/mL	3.5	Nd	27.2
ALP, U/L	177	241	203
Uric Acid, mg/dL	5.2	5.8	5
Fasting Blood Glucose, mg/dL	93	80	83
Hemoglobin, g/dL	10.3	17.9	14.8
Urine PH	6	7	7
Urine Calcium 24h, mg	Nd	600	700
Serum Creatinine After Renal Transplantation, mg/dL	1.1	No Kidney Transplant	No Kidney Transplant
Serum Magnesium After Treatment with Magnesium Tablet, mg/dL	2	2.05	1.92

Abbreviations: eGFR, estimated glomerular filtration rate calculated by Cockcroft-Gault equation; ALP, alkaline phosphatase; PTH, parathyroid hormone; Nd, not determined.

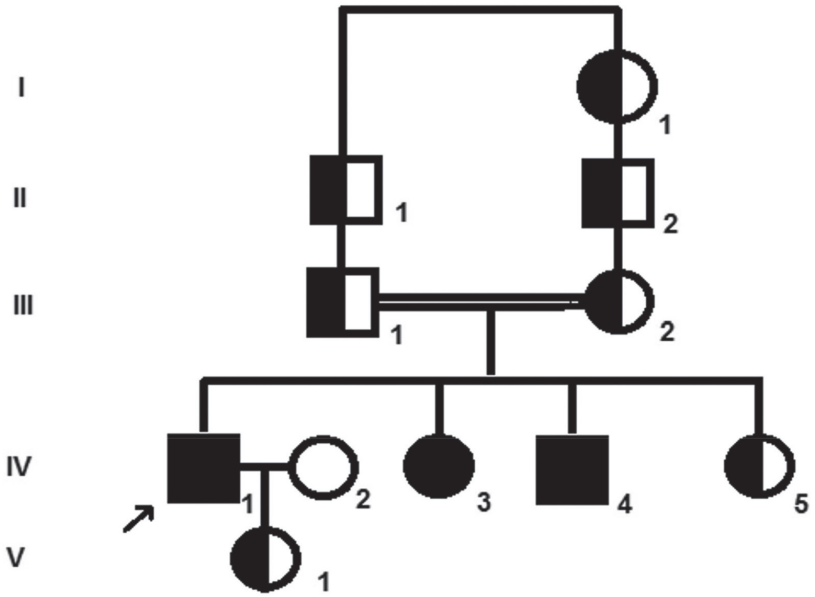

Figure 1. Pedigree of the Family (The black arrow shows the patient with ESKD. p.C113Y mutation in *CLDN16* gene with homozygote condition was found in IV-1, IV-3, and IV-4). I-1, II-1, II-2, III-1, III-2, IV-5, and V-1 are obligate carriers.

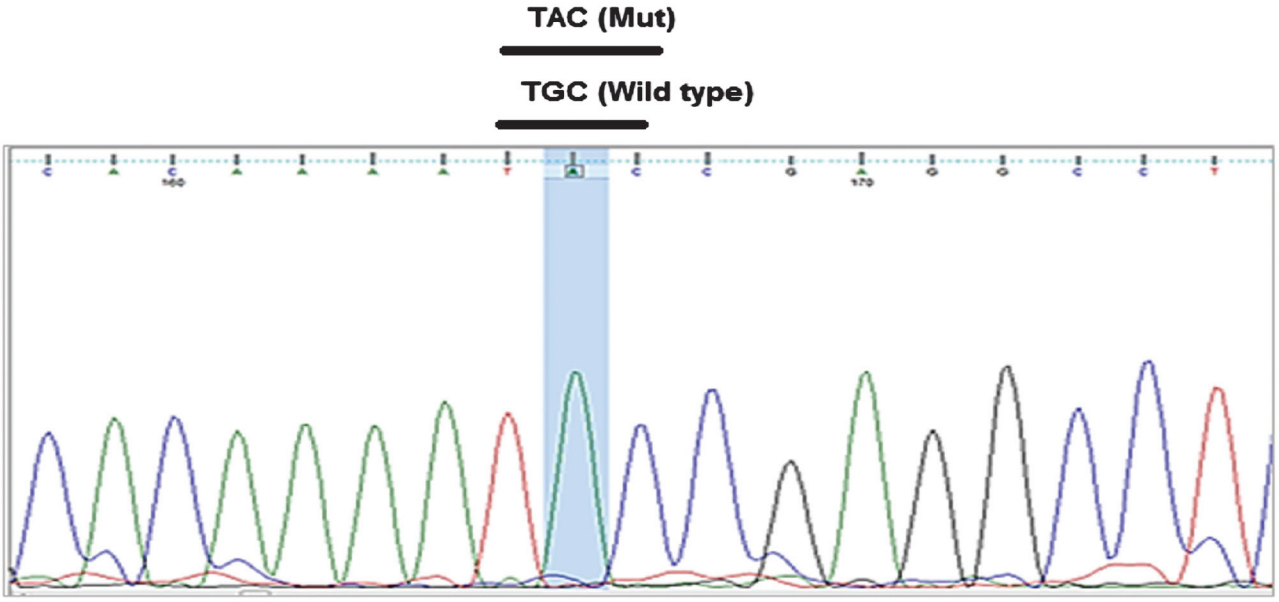

Figure 2. Sanger sequencing result showed c.338G > A: p.C113Y in exon 2 of CLDN16 gene. blue line shows homozygosity.

The study of Geeta Hampson *et al.* described two FHHNC patients with *CLDN16* gene mutation who underwent kidney transplantation at an earlier age compared to the patient in our study.[18] Therefore, it is probable that the age of onset and the severity of the kidney disease could be predicted by the patient genotype.[13]

In another study, a 33-year-old Chinese woman with FHHNC was evaluated and a novel missense mutation (c.346C > G, p. Leu116Val) was found in *CLDN16*.[19] The patient developed ESKD at the same age as our patient.

A recent study from India reported a new homozygous nonsense mutation in *CLDN16* (c.620G > A, p. Trp207Ter) in two children.[20] Müller *et al.* published a paper in which a child with Iranian parents was diagnosed with FHHNC. According to their study, the mutation was found to be in *CLDN16* T233R.[21]

CONCLUSION

Overall, it seems that patients with Middle Eastern and Asian ancestry are more likely to have CLDN*16* gene mutations compared to *CLND19* mutations. Patients with *CLND19 and CLDN16* gene mutations develop CKD and eventually ESKD in 61% and 33% of cases respectively.[1] Therefore, these patients might have a slower rate of progression to ESKD, although more studies are needed.

We recommend more detailed genetic evaluations in young patients with ESKD and nephrocalcinosis as the disease might remain undiagnosed without genetic analysis.

STATEMENT OF ETHICS

The study was approved ethically by the ethical committee of human studies of Iran University of Medical Sciences under the registered number of IR.IUMS.FMD.REC.1399.640. Written consent was obtained with permission for publishing the clinical information of the patients.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

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