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증 례

선천성 염소성 설사를 가진 환아에서 국소 분절 사구체경화증이 발생하여 만성 신장병으로 발전한 사례

한림대학교 의과대학 춘천성심병원 소아청소년과

서영준 · 정한빈 · 안석민 · 신우철 · 배은주 · 윤종형 · 정활림 · 이홍진

A Case of Progressive FSGS and Chronic Kidney Disease in Congenital Chloride Diarrhea with *SLC26A3* Mutation

Young-Jun Seo, Han Bin Cheong, Seok Min An, Woo Cheol Sin
Eun Joo Bae, Jong Hyung Yoon, Hwal Rim Jeong, Hong Jin Lee

Department of Pediatrics, Hallym University Chuncheon Sacred Heart Hospital, Chuncheon, Gangwondo, Republic of Korea

We present the case of long-term observation of a patient with chronic kidney disease (CKD) caused by advanced focal segmental glomerulosclerosis (FSGS) resulting from underlying congenital chloride diarrhea (CLD). A 20-year-old woman was admitted for prolonged proteinuria despite conservative treatment for CLD. She was diagnosed with CLD and started taking KCl salt supplementation from the time of birth. Mild proteinuria was first found at 12 years of age, which progressed to moderate proteinuria at 16 years of age. At 16 years of age, CKD stage 2 with FSGS was diagnosed based on the initial assessment of the glomerular filtration rate (GFR) and kidney histology. On admission, we re-assessed her renal function, histology and genetic analysis. GFR had deteriorated to CKD stage 4 and renal histology revealed an advanced FSGS combined with tubulointerstitial fibrosis. A homozygous mutation in the *SLC26A3* gene (c.2063-1G>T) was found by diagnostic exome sequencing and may have been inherited from both parents. CLD patients can be more vulnerable to renal injury, which may also cause progression of renal failure. Therefore, even if there is an early diagnosis and adequate salt supplementation, close monitoring of renal function and tailored treatment should be emphasized for renal protection and favorable CLD prognosis.

Key words: Congenital chloride diarrhea, Focal segmental glomerulonephritis, Chronic kidney disease

Introduction

Congenital chloride diarrhea (CLD) is a rare autosomal recessive inherited disorder that results from a mutation of solute carrier family 26 member 3 (*SLC26A3*) on the minus strand of chromosome 7q31.1 (Online Mendelian Inheritance in Man: 126650 and 214700)[1]. *SLC26A3* is a Cl^-/HCO_3^- exchanger that affects apical epithelial Cl^- absorption and HCO_3^- secretion, secondary absorption of Na^+, and secretion of H^+ through coupling to the Na^+/H^+ exchanger in the intestinal epithelium[2]. Mutation of *SLC26A3* causes disruption of Na^+ and Cl^- reabsorption in the intestinal epithelium resulting Cl-rich diarrhea, hy-

*The study was approved by the institutional review board (IRB) and the consent was waived due to the nature of the retrospective study.

책임저자: 이홍진, 강원도 춘천시 삭주로 77
한림대학교 춘천성심병원 소아청소년과
Tel: 033)240-5802, Fax: 033)241-8063
Email: hjlee@hallym.or.kr

pochloremia, hyponatremia, hypokalemia, metabolic alkalosis, and dehydration.

Approximately 250 cases of CLD have been reported worldwide and some countries have a higher incidence such as Finland (1:30,000-40,000), Poland (1:200,000), and some other middle-eastern Asian countries including Kuwait and Saudi Arabia (1:3,200-1:5,000 of regional estimate) where consanguineous marriage is allowed[3]. However, CLD is extremely rare in Korea and only a few cases have been reported to date. Although the long-term prognosis of patients with CLD was relatively favorable with abundant salt supplementation treatment, the incidence of renal impairment in a long-term follow-up period has not been estimated in Korea. One previous study reported that one of five investigated pediatric CLD patients showed an increased BUN/creatinine value and abnormal kidney ultrasound findings at 14 months of age[4]; however, there was no information about genetic analysis. One patient with FSGS was reported recently, and this patient had renal complications that resulted from an activated renin-angiotensin system because of CLD and concomitant congenital renal dysplasia, but no other long-term follow-up has been reported[5]. We report the single-center long-term follow up data from a CLD patient who developed CKD stage 4 resulting from FSGS, and the diagnostic exome analysis of the patient and her family.

Case Report

A 20-year-old woman, who had been taking KCl salt supplementation for CLD and who was previously diagnosed with FSGS at 16 years of age, was admitted for evaluation of renal insufficiency. She was referred from the department of orthopedics before exploratory surgery for left ankle pain, which developed 1 year before referral and was worse when she was walking. MRI findings showed suspicious pigmented villo-nodular synovitis. The patient complained of mild fatigue, frequent diarrhea, and left ankle pain, but no other symptoms.

She was born via cesarean section at 36+2 weeks gestation, weighed 3.5 kg, and had polyhydroamnios. Her Apgar score was 7 at 1 minute and 8 at 5 minutes. The patient was suspected of having CLD or other congenital intestinal problems before birth because generalized fetal bowel dilatation with polyhydroamnios showed on a prenatal ultrasound test. Frequent watery diarrhea was observed since birth and her first hospitalization was at day 9 after birth because of lethargy and diarrhea. The patient started KCl salt supplementation during the neonatal period. CLD was confirmed by a stool test, in which the fecal electrolytes were Cl^-, 90 mmol/L; K^+, 21 mmol/L; and Na^+, 78 mmol/L. During the infant and toddler periods, she was hospitalized six times for moderate dehydration with electrolyte imbalance, but no other episode occurred in the child to early adolescent period. She remained in generally good condition and developed well based on regular check-up records (Fig. 1).

In January, 2013, at age 16 years, the patient was referred for proteinuria ≥3+ on a dipstick test. The in-hospital evaluation showed that proteinuria was 0.45 g/24 h (0.404 g/24 h/m^2), serum albumin was 3.8 g/dL, and the estimated glomerular filtration rate (GFR) was 72.88 mL/min/1.73 m^2 using the Schwartz formula. Blood pressure and serum electrolyte values were within normal range but kidney ultrasound revealed increased renal parenchymal echogenicity in both kidneys. In the kidney biopsy, renal parenchymal global sclerosis was identified in one glomerulus among

seven retrieved glomeruli, which suggested segmental sclerosis. The glomerular mesangial matrix also showed a focal patch of tubular atrophy and mild interstitial lymphocyte infiltration (Fig. 2A).

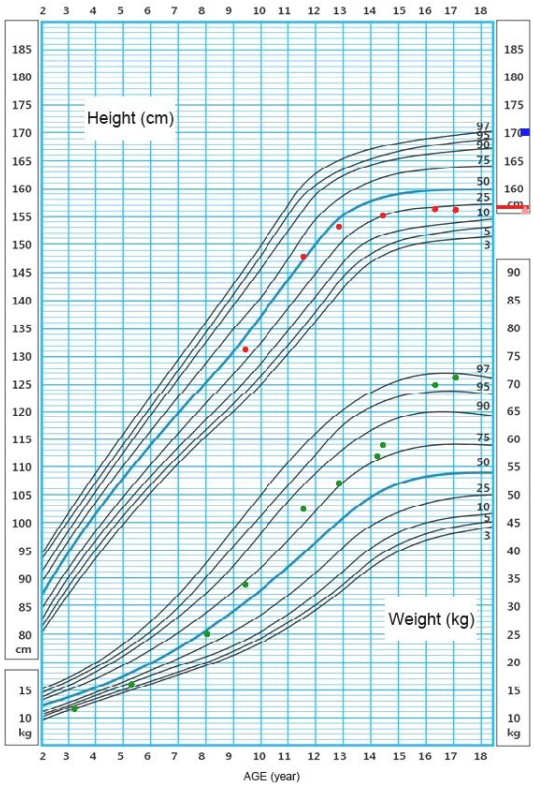

Fig. 1. Growth velocity of our patient.

However, glomerular basement membranes appeared normal in thickness (Fig. 2B). Immunofluorescence analysis showed nonspecific deposits for IgM and C3. Electron microscopy revealed that glomerular capillary endothelial cells were swollen and obliterated fenestrae, and there was diffuse effacement of the visceral epithelial cell foot processes (Fig. 2C). In a follow-up evaluation after 3 months, proteinuria worsened to ≥4+ on the dipstick test, and oral prednisolone 60 mg/day (1 mg/kg/day) was initiated and continued for 3 months. Proteinuria then improved to 2+ by the dipstick test, after which the steroid therapy was tapered and stopped. Proteinuria persisted at 2+ by the dipstick test, which was estimated as 1.81 g/24 h in the follow-up evaluation at 3 months after prednisone withdrawal. For the next 3 years during the outpatient follow-up period, the patient had taken no other medication for proteinuria except salt supplementation, but sustained proteinuria ≥3+ remained, and the patient was in a relatively good general condition.

On admission, we examined the patient's physical findings, complete blood count (CBC), routine blood chemistry, urinalysis, assessment of GFR,

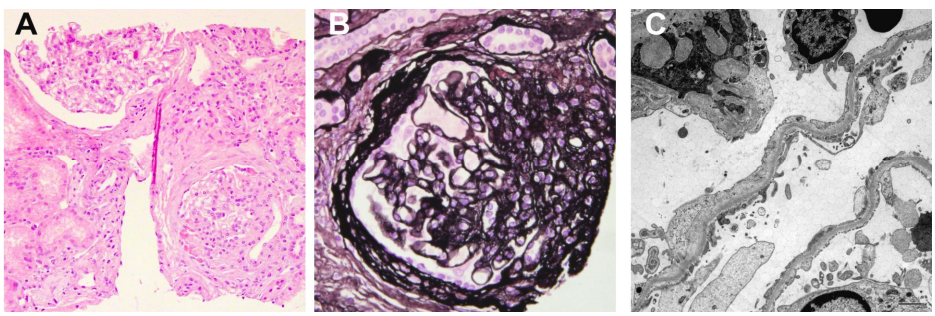

Fig. 2. Kidney histology of FSGS, a biopsy at patient 16 years old. (A) Light microscope image of glomeruli shows an increase in size but normal cellularity with mild variable mesangial matrix expansion. There is also focal patch tubular atrophy and mild interstitial lymphocytes infiltration (H&E, ×200). (B) Silver staining shows that the glomerular basement membranes appear normal in thickness, contour, and texture (silver staining, ×400). (C) An electron micrograph (EM) image reveals that most of the glomerular capillary lumen are patent with mild mesangial expansions but glomerular capillary endothelial cells are swollen and obliterate the fenestrae. Visceral epithelial cells show diffuse effacement of foot processes on about 70% of the external capillary surfaces (scale bar=5 μm).

and secondary kidney biopsy. She was obese (body mass index, 33.41; height, 154 cm; weight, 86.1 kg), but her blood pressure was normal (120/80 mmHg) without other findings associated with chronic kidney disease. In laboratory tests, the patient's CBC count was unremarkable: WBC, 7,870/μL (seg 68.7%); hemoglobin, 12.7; and platelets, 459,000/μL. The serum Na^+ level was within the normal range at 143 mmol/L, K^+ was 3.5 mmol/L, and Cl^- was 99 mmol/L, but serum uric acid was increased, at 14.8 mg/dL. The patient's medical records revealed that hyperuricemia was first observed at 7 months of age and intermittently recurred through the toddler years to school age, ranging from 9.0–15.0 mg/dL. Serum protein and albumin levels were within the normal range at 7.2 and 3.7 g/dL, respectively. The patient's serum blood urea nitrogen level was increased at 51.2 mg/dL and the patient's kidney function was decreased, with a serum creatinine (Cr) level of 3.7 mg/dL, and an estimated GFR of 18.92 mL/min/1.73 m^2. Serum 25(OH) vitamin D and parathyroid hormone were 13.1 ng/mL and 250.5 pg/mL, respectively. Serum calcium, phosphate, and alkaline phosphatase levels were 8.8 mg/dL, 4.5 mg/dL, and 90 IU/L.

Urinalysis showed proteinuria (4+/4+), but was otherwise within the normal range. Twenty-four-hour collected urine chemistry showed that the urine calcium level was 4 mg/day. Renal ultrasound showed that both kidneys were in chronic renal failure (right and left kidneys were approximately 8 cm), and a small cyst (approximately 1 cm) was found in the right kidney. Kidney biopsy revealed diffuse tubular atrophy with interstitial fibrosis (Fig. 3A). Immunofluorescence showed a few granular deposits for C3 (1+) but the results were negative for other markers such as IgG, IgA, IgM, C1q, C4, Kappa, Lambda, and fibrinogen (Fig. 3B). On electromicroscopic findings, glomerular basement membranes (GBMs) showed some large electron-dense deposits (hump) in subepithelial locations and some in intramembranous and mesangial locations (Fig. 3C). These results overall suggested advanced FSGS and tubulointerstitial findings.

To clarify affected mutation of *SLC26A3* gene as well as other possible genetic predisposition to

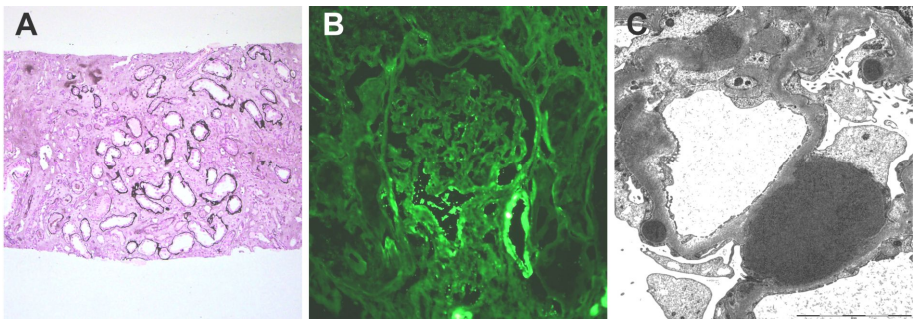

Fig. 3. Histologic examination of the patient's second renal biopsy at patient 20 years of age. (A) Silver-stained section reveals medullar portion without glomerulus. Tubules show diffuse atrophy with interstitial fibrosis (silver staining, ×100). (B) Sections for the immunofluorescence (IF) study reveal a few granular deposits for C3 (1+) with glomerular hypertrophy, diffuse tubular atrophy, and dilatations containing cast (IF, ×400). (C) EM image showing that most of the glomerular capillary lumen are partially compressed by mesangial expansions. Glomerular basement membranes show some large electron-dense deposits (hump) in subepithelial locations and some in intramembranous and mesangial locations. Visceral epithelial cells show partial effacement of the foot process on about 30% of the external surface (scale bar=5 μm).

CKD in CLD, diagnostic exome sequencing was conducted using Nextseq (Illumina, San Diego, CA) and the TruSight one-sequencing panel capturing 4,813 genes and 62,000 exons (Greencross Medical Genetics Co.). Sequencing analysis data revealed that the patient had a splice site mutation in intron 18 (c.2063-1G>T) of the *SLC26A3* gene, which has been listed in the Korean Mutation Database (https://kmd.nih.go.kr/kmd/search?type=geneid&query=NM_000111.2).

A family study showed that her mother and one of elder sibling were heterozygous healthy carriers with a splice site mutation (c.2063-1 G>T). The patient's father was not assessed because he died in car accident at approximately 40 years of age; he had no underlying diseases (Fig. 4).

The patient was discharged after a diagnostic work-up and was recently followed-up monthly at the out-patient clinic. She has started to take additional medications including xanthine oxidase inhibitor (febuxostat, 40 mg/day) for hyperuricemia and calcitriol (0.25 μg/day) for suspected vitamin D insufficiency. An exploratory surgery for her left ankle was postponed, because the ankle pain was relatively well-controlled by intermittent analgesics and surgery was not considered to be critical considering her general condition.

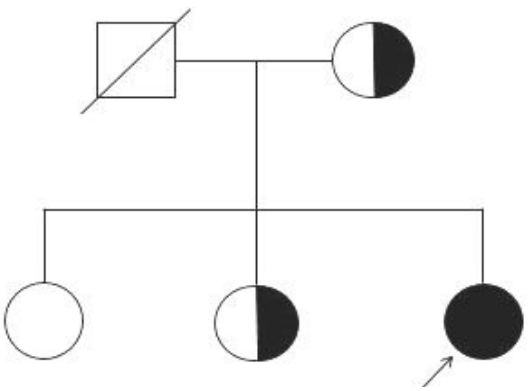

Fig. 4. Pedigree analysis of the patient's *SLC26A3* gene (c.2063-1G>T mutation).

Discussion

Renal complications are frequently accompanied by CLD[6-8]. According to one Finnish study, CKD develops in approximately 28% of CLD patients (10 of 35 investigated patients) during an average of 27.6 years of observation, and nephrocalcinosis was the major comorbid finding with CKD[7]. The nephrocalcinosis pathophysiology seems to be independent of *SLC26A3* mutations because of recurrent nephrocalcinosis in transplanted kidneys in CLD patients[9], but other congenital kidney problems such as congenital nephrotic syndrome, renal hypoplasia, and dysplasia have also been suggested to be involved in renal injury in CLD patients[6]. It is unclear whether congenital abnormalities occur because of genetic mutations in *SLC26A3* or secondary to the generalized growth failure. However, major risk factors for CKD in childhood are consistent with congenital renal abnormalities, and they have been considered to be contributing factors to renal injury in CLD patients.

In this report, we present the case of a CLD patient with a homozygous mutation of c.2063-1G>T in the *SLC26A3* gene, who also had advanced FSGS and CKD stage 4 that was identified through serial kidney evaluations. A family study revealed that her mother and one elder sister were heterozygous carriers of the splice site mutation. Parental genotyping could not be completed, because the patient's father died at 40 years of age in a car accident. However, a heterozygous mutation in one elder sibling suggested that both parents might be heterozygous carriers and the patient's genetic mutation was likely inherited from her parents.

Early suspicion and prompt diagnosis such as an antenatal ultrasound screening were emphasized in Finland, where CLD is the most prevalent in

the world, because early sufficient salt substitution could improve clinical outcomes[10]. Our patient was also suspected of having CLD at the antenatal ultrasound screening test, which was confirmed in the neonatal period and this result caused initiation of early disease management and salt supplementation (KCl 1-2 mmol/kg/day). The possible cause for renal injury might be the KCl-only supplement that is no longer recommended because of the risk of chronic volume contraction causing renal impairment and growth retardation [10]. In this case, we cannot rule out the chronic electrolyte deficit, which may have resulted in renin-angiotensin-aldosterone system activation, thereby exacerbating an injury cascade that leads to FSGS progression. As described above, the patient's medical records showed that she had several hyperuricemia events since 10 months of age, with a range between 9.0 to 15.0 mg/dL. Hyperuricemia may occur because of a reduction in the renal urate clearance secondary to high angiotensin activity, which returns to normal with adequate control, and this could be additional evidence that supports electrolyte insufficiency. Uric acid results in pro-oxidative effects and subsequent deleterious effects in renal tissues, and thus, it might exacerbate renal failure in our patient. Additionally, blockade of the renin-angiotensin-aldosterone system using an angiotensin converting enzyme inhibitor or angiotensin receptor blocker should be beneficial at slowing the progression of kidney disease, but these management strategies were not applied, which also might result a relatively faster rate of GFR decline after our patient was diagnosed with FSGS.

On the other hands, salt and volume deficits may have not been serious enough to cause growth retardation or nephrocalcinosis in our case. Because the average Korean dietary sodium is approximately 1.5 g/day higher than other western-European countries or the United States, according to a survey of global, regional, and national sodium intake in 1990 and 2010, we speculate that the reason may be because of the patient's extra sodium intake. Additionally, at the out-patient follow-up visits, it was recommended to our patient that she should have a salty diet for extra sodium intake, which may explain why she showed less salt deficiency.

The c.2063-1G>T mutation is a splicing mutation in the acceptor site of intron 18, and it has been suggested to be the likely cause of a splicing error and the loss of function of the *SLC26A3* protein[11]. Recently, characterization of the *SLC26A3* gene mutation has been reported in eight Korean CLD patients, in whom c.2063-1G>T was the most common mutation, in four of the eight patients[12]. Kim et al. previously reported a rare case of a secondary FSGS patient with CLD who had the c.2063-1G>T mutation[5]. The author described a 7-year-old boy who had mild proteinuria since 10 months of age but who maintained normal kidney function at the time of renal biopsy. This seems similar to our patient's renal histology and genetic background, and one important finding was the patient had congenital renal dysplasia, which may, on its own, initiate or promote kidney disease.

Low-level *SLC26A3* expression in the kidney was shown to play a minor role in regulation of acid-base and NaCl balance[13], but differential disease characteristics such as male subfertility or drug response to diarrhea-modulating agents depends on the *SLC26A3* mutation variant[14,15]. Although the leading cause for patients' FSGS might be functional maladaptation to renal stress such as insufficient salt replacement and a subsequent increase in glomerular pressure and ca-

pillary flow rate, the severity of renal involvement could depend on genotypic-phenotypic differences. However, the differential renal involvement according to variant gene mutations has not been investigated, and whether the c.2063-1G>T variant is likely contribute to renal insufficiency in CLD patients should be investigated in future studies.

Although considerable improvement in CLD management has decreased the mortality rate and early hospitalization for severe dehydration and electrolyte deficit, chronic complications and the long-term prognosis of patients on salt replacement therapy are still important issues. Patients with the same genotype may show different kidney complications throughout their life, and therefore sufficient salt substitution as well as a regular follow-up of kidney function should be emphasized in all CLD patients to prevent CKD.

요 약

선천성 염소성 설사를 가진 환아에서 국소 분절 사구체경화증이 발생하여 말기 신장병으로 발전한 사례를 보고 하고자 한다. 20세 여자 환자로, 본원에서 출생 전 산전진단에서 양수과다 및 초음파 소견으로 선천성 염소성 설사가 의심되었으며, 출생 직후 확진 되어 신생아기 때부터 KCl 보충을 통하여 증상 조절을 시작하였다. 환아는 이후 특별한 건강의 문제가 없었으나 12세에 단백뇨가 관찰되었고, 16세때 본원에서 국소분절 사구체경과증 과 2기 만성신장병 진단을 받았다. 이후 보존적 치료를 하였으며, 지속적인 단백뇨에 대한 재 평가를 위하여 입원하게 되었다. 입원 후 확인된 검사에서 사구체여과율(GFR)은 4기 신장병으로 악화되어 있었으며 신생검에서도 국소분절 사구체신염으로 인한 만성 신장병이 재 확인 되었다. 환아 및 가족을 대상으로 시행한 유전자 검사(diagnostic exome sequencing)에서는 *SLC26A3* 유전자의(c.2063-1G>T) 동형 접합체 변이가 각각 부모에서 전달된 것을 확인하였다. 선천성 염소성 설사 환자는 적절한 전해질 보충에도 불구하고 신기능 손상이 되기 쉬운 경향이 있으며, 따라서 조기 진단 및 충분한 전해질 보충이 이루어지는 경우에서도 환자의 신장 기능에 대한 정기적 관찰 및 적절한 보조 치료가 필요할 것으로 사료된다.

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