Clinical Features, Molecular Genetics, and Long-Term Outcome in Congenital Chloride Diarrhea: A Nationwide Study in Japan

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Objective To clarify clinical and genetic features of Japanese children with congenital chloride diarrhea (CCD).

Study design This was a multi-institutional, retrospective survey of 616 pediatric centers in Japan with identified patients with CCD between 2014 and 2018. Mutations involving *SLC26A3* were detected by Sanger sequencing.

Results Thirteen patients met all entry criteria including mutations in *SLC26A3*, and 14 patients satisfied clinical diagnostic criteria. Homozygous or compound heterozygous mutations in *SLC26A3*, including 6 novel mutations, were identified in 13 of these 14 patients (93%). The most common (detected in 7 of 13) was c.2063-1g>t. Median age at diagnosis was 1 day. Nine of the patients meeting all criteria were diagnosed as neonates (69%). Median follow-up duration was 10 years. When studied, 8 patients had <5 stools daily (62%), and all had fewer than in infancy. Only 1 patient had nephrocalcinosis, and 3 (23%) had mild chronic kidney disease. Neurodevelopment was generally good; only 1 patient required special education. Five patients (38%) received long-term sodium, potassium, and chloride supplementation.

Conclusions Early fetal ultrasound diagnosis and prompt long-term sodium, potassium, and chloride supplementation were common management features. Genetic analysis of *SLC26A3* provided definitive diagnosis of CCD. In contrast with previously reported localities, c.2063-1g>t might be a founder mutation in East Asia. *(J Pediatr 2019;214:151-7)*.

Congenital chloride diarrhea (CCD; MIM# 214700) is a rare autosomal-recessive disorder caused by mutations in the solute carrier family 26 member 3 (*SLC26A3*) gene, which encodes an intestinal $Cl^-/HCO3^-$, Na^+-independent exchanger.[1,2] Intrauterine onset manifests as polyhydramnios, dilated fetal bowel loops, and lack of infant meconium,[3] findings mimicking intestinal obstruction, sometimes leading to unnecessary surgery.[4]

Children with CCD often are born premature and, soon after birth, profuse diarrhea leads to dehydration, hypochloremia, hypokalemia, metabolic alkalosis, and failure to thrive. Diagnosis of CCD involves watery diarrhea soon after birth and excessive fecal chloride. Misdiagnosis of Bartter syndrome may occur when watery diarrhea is unnoticed or misinterpreted as urine.[5,6] Although untreated CCD causes early death from impaired renal function, nephrocalcinosis, and end-stage renal disease,[7] oral salt replacement with sodium chloride (NaCl) and potassium chloride (KCl) allows normal growth and development, with favorable long-term outcome.[3,8,9] Prompt diagnosis and replacement therapy are essential.

ACMG	American College of Medical Genetics and Genomics	KCl	Potassium chloride
BMI	Body mass index	NaCl	Sodium chloride
CCD	Congenital chloride diarrhea	PPI	Proton pump inhibitor
CKD	Chronic kidney disease	*SLC26A3*	Solute carrier family 26 member 3
eGFR	Estimated glomerular filtration rate	STAS	Sulfate transporter and anti-sigma antagonist
HGVD	Human Genetic Variation Database	UCSC	University of California, Santa Cruz

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Supported by the Japanese Ministry of Health, Labour, and Sciences (Research Grants for Research on Intractable Disease; H24-037, H26-045, and H29-015, to T.T.). The authors declare no conflicts of interest.

https://doi.org/10.1016/j.jpeds.2019.07.039

Clinical and genetic findings of CCD have been reported mostly from 3 high-incidence areas: Finland, Poland, and Saudi Arabia[10-12] (The Human Gene Mutation Database in January 2015; http://www.hgmd.org/; BIOBASE, Waltham, Massachusetts). One single-center study[13] and a few cases[14,15] have been reported from East Asia, so whether East Asian patients clinically or genetically differ from others remains unclear. We conducted a nationwide study of Japanese children with CCD, focusing on clinical features and *SLC26A3* mutations.

Methods

This retrospective observational and prospective genetic analysis was conducted within the framework of the Nationwide Survey of Congenital Diarrheas and Enteropathies in Japan, established in 2014 by a study group seeking guidelines for managing intractable pediatric gastrointestinal diseases. Study protocols complied with the ethical guidelines of the Declaration of Helsinki (2013 revision). The nationwide survey and the genetic analysis protocols respectively were approved by the ethics committees at Osaka Women's and Children's Hospital and at Kurume University. Written informed consent was obtained from enrolled patients or their parents.

Nationwide Questionnaire Survey

Questionnaires were mailed to 616 Japanese pediatric centers. This initial survey asked whether centers had encountered patients with 20 congenital diarrheas and enteropathies between January 2005 and December 2014, including CCD. The second questionnaire survey, sent to centers initially reporting CCD, asked about fulfilment of clinical diagnostic criteria; fetal, infantile, and recent clinical and laboratory findings; and results of molecular analyses involving *SLC26A3*. Clinical diagnosis was based on high fecal chloride (>90 mmol/L) and persistent watery diarrhea beginning soon after birth.

Genetic Analysis of *SLC26A3*

For detection of *SLC26A3* mutations, genomic DNA was isolated from peripheral blood cells from each patient and participating parent. DNA was subjected to polymerase chain reaction using primers listed in **Table I** (available at www.jpeds.com). Primer sets were designed to amplify 21 exons including the 5′-untranslated region and the coding regions including exon–intron boundaries. Polymerase chain reaction products were treated with ExoSAP-IT Express PCR Cleanup Reagents (Thermo Fisher Scientific, Waltham, Massachusetts) to inactivate free primers and dNTPs, and then subjected to sequencing reactions using forward or reverse primers and BigDye Terminator v3.1 (Thermo Fisher Scientific). DNA fragments were purified using Centri-Sep spin columns (Princeton Separations, Princeton, New Jersey). Sequencing was carried out with an ABI 3100 Genetic Analyzer (Applied Biosystems, Waltham, Massachusetts).

Sample sequences were aligned to reference sequences obtained from the University of California, Santa Cruz (UCSC) Genome Bioinformatics Web site (http://genome.ucsc.edu/index.html) using the ClustalW program (https://www.genome.jp/tools-bin/clustalw) to identify nucleotide changes. Mutations were numbered according to GenBank Reference Sequence NM_000111. Names of identified variants were assigned following guidelines from the Human Genome Variation Society (version 2; http://www.hgvs.org/mutnomen/). Chromosomal coordinates were assigned according to the GRCh37/hg19 assembly. The Human Gene Mutation Database (January 2015; http://www.hgmd.org/), Sorting Intolerant Form Tolerant (http://sift.jcvi.org/), and MutationTaster2 (http://www.mutationtaster.org/) were used as computational predictive programs. Minor allele frequency was referred to the UCSC Genome Browser and the Japanese data set of the Human Genetic Variation Database (HGVD; http://www.hgvd.genome.med.kyoto-u.ac.jp/). American College of Medical Genetics and Genomics (ACMG) interpretation guidelines were followed in assessing pathogenicity of detected variants.[16]

Clinical and Laboratory Findings

Clinical and laboratory findings were analyzed for patients in whom mutations of *SLC26A3* were identified. Perinatal observations as well as clinical and laboratory findings at diagnosis, during infancy, and at most recent follow-up were obtained retrospectively from medical records. Perinatal observations included date of birth, sex, gestational duration, birth weight, presence or absence of polyhydramnios and dilated fetal bowel loops on fetal ultrasound scan, and any fetal diagnoses. Clinical and laboratory data at diagnosis included age; watery diarrhea; abdominal distention; fecal chloride content; serum sodium, potassium, and chloride concentrations; and treatments during infancy. Features representing long-term outcome included follow-up duration; fecal characteristics; number of stools; serum sodium, potassium, and chloride; estimated glomerular filtration rate (eGFR); 24-hour urinary sodium, potassium, and chloride; treatments; growth; education and employment; presence or absence of soiling; and complications and problems. Number of stools per day was evaluated as 1-4, 5-9, or >9. Mild and moderate-to-severe chronic kidney disease (CKD) were defined as eGFR from 60 to 89 mL/min/1.73 m^2 or <60, respectively.[17,18] Growth was evaluated by height, weight, and body mass index (BMI), all expressed in terms of SD.[19] Recent soiling was evaluated in patients ≥6 years old.

Results

The initial survey sent to 616 pediatric centers yielded replies from 529 (86%) between 2014 and 2015, with information

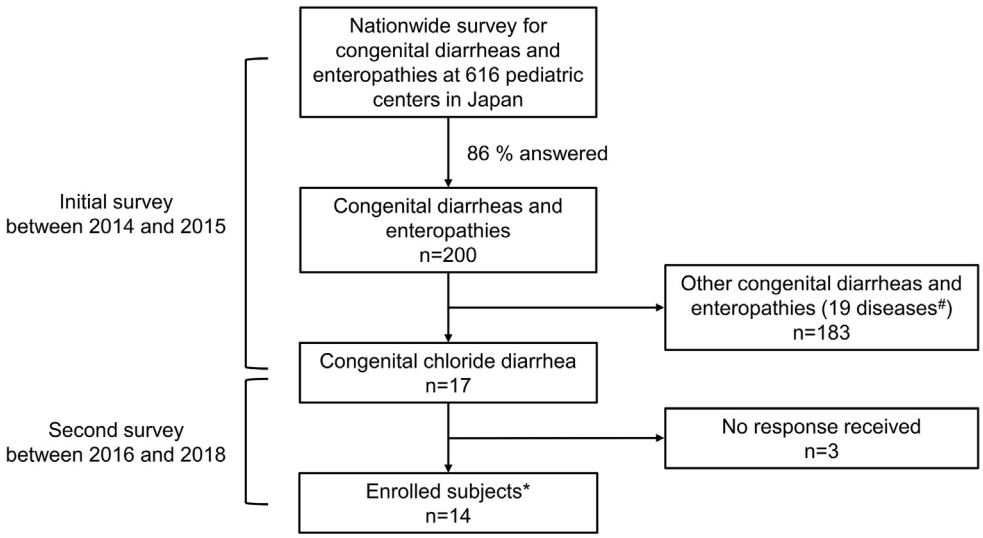

Figure 1. Flow chart of this nationwide survey. *Clinical diagnostic criteria for CCD in this study were high fecal chloride concentration (>90 mmol/L) and persistent watery diarrhea beginning soon after birth. #The other 19 diseases included Shwachman–Diamond syndrome, congenital sucrase-isomaltase deficiency, congenital lactose intolerance, enterokinase deficiency, pancreatic lipase deficiency, glucose–galactose malabsorption, congenital sodium diarrhea, fructose malabsorption, abetalipoproteinemia, microvillus inclusion disease, tufting enteropathy, primary intestinal lymphangiectasia, autoimmune enteropathy, immunodysregulation, polyendocrinopathy and enteropathy X-linked (IPEX) syndrome, celiac disease, vasoactive intestinal peptide-secreting tumor (VIPoma), multiple endocrine neoplasia, infant intractable diarrhea, and mitochondrial respiratory chain disorders.

concerning 200 patients with congenital diarrheas and enteropathies including 17 with CCD. In the second survey sent to the 14 centers encountering CCD, we received detailed information between 2016 and 2018 for 14 CCD patients from 11 centers. We enrolled these patients in our study (**Figure 1**). All were Japanese. Two had undergone genetic analysis of *SLC26A3* at their centers, including 1 reported previously.[14] We performed genetic analysis at Kurume University for the remaining 12 patients.

Genetic Features

We identified 12 different sequence alterations including 7 missense mutations (c.358G>T, c.358G>A, c.392C>T, c.392C>G, 1043T>A, c.1198T>C, and c.1644C>G), 3 splice-site mutations (c.888+1g>a, c.1677+1g>c, and c.2063-1g>t), and 2 truncating insertion/deletions (c.1007_1008insT and c.1342_1343del) in *SLC26A3* in 13 of 14 patients (93%), including 1 previously reported (#9).[14] One patient showed no mutation (#14; **Table II**). Among mutations detected, 6 were unreported previously: c.358G>T, c.888+1g>a, c.1043T>A, c.1198T>C, c.1644C>G, and c.1677+1g>c (**Figure 2**; available at www.jpeds.com). None of these were registered previously in genome databases, including the UCSC genome browser, HGVD, and Human Gene Mutation Database. Three patients had homozygous aberrations (numbers 1, 3, and 13), and the remaining 10 patients had compound heterozygous abnormalities. The most common mutation was c.2063-1g>t (detected in 7 patients). We also examined available DNA from parents of 7 patients; all were carriers (**Table II** and **Figure 3** [available at www.jpeds.com]). All 12 mutations identified were classified as pathogenic or possibly pathogenic according to ACMG standards and guidelines.[16] Characteristics of these *SLC26A3* variants are summarized in **Table II**.

Patient Characteristics During the Perinatal Period and Infancy

Perinatal information for 4 patients (#4, 9, 10, and 14) was published previously.[14,20-22] **Table III** (available at www.jpeds.com). summarizes perinatal findings, clinical and laboratory features at diagnosis, and treatment during infancy for the 13 patients with mutations of *SLC26A3*. Three, 1, 3, and 6 patients were born in the 1970s, 1990s, 2000s, and 2010s, respectively. Seven were male and 6 were female. Median gestational duration and birth weight were 36 weeks (range, 28-38) and 2640 g (range, 970-3156), respectively. Eight patients (62%) were premature (before 37 weeks), and 5 (38%) had low birth weight (<2500 g), including 1 with extremely low birth weight (<1000). Median age at diagnosis was 1 day (range, first day to 8 months); 9 patients (69%) were diagnosed while neonates. Fetal ultrasound scan showed both polyhydramnios and dilated fetal bowel loops in 9 patients (69%). Three patients (#1, 2, and 3) showed only polyhydramnios with no information about dilated fetal bowel loops because ultrasound scan was performed in the 1970s. One patient (#8) had no fetal ultrasound scan because he was born at extremely low birth weight following premature rupture of membranes. Six (46%) and 3 patients (23%), respectively,

Table II. Characteristics of detected variants in *SLC26A3*

Patients	Exon/intron	Nucleotide change	Predicted amino acid change	Mutation type	Classified sequence variants	HGMD	Mutation taster	SIFT	Frequency (%)*	Parental origin
1	Exon 15	c.1644C>G	p.N548K	Missense	Likely pathogenic	–	Disease causing	Damaging	–/–	Not determined
	Exon 15	c.1644C>G	p.N548K	Missense	Likely pathogenic	–	Disease causing	Damaging	–/–	Not determined
2	Exon 5	c.392C>G	p.P131R	Missense	Likely pathogenic	DM	Disease causing	Damaging	0.001/–	Not determined
	Intron 18	c.2063-1g>t	Intron acceptor site AG loss	Splice-site change	Pathogenic	DM	–	–	0.006/–	Not determined
3	Intron 18	c.2063-1g>t	Intron acceptor site AG loss	Splice-site change	Pathogenic	DM	–	–	0.006/–	Paternal
	Intron 18	c.2063-1g>t	Intron acceptor site AG loss	Splice-site change	Pathogenic	DM	–	–	0.006/–	Maternal
4	Exon 12	c.1342_1343del	p.Leu448Lysfs*9	Deletion	Pathogenic	DM	–	–	–/–	Paternal
	Intron 18	c.2063-1g>t	Intron acceptor site AG loss	Splice-site change	Pathogenic	DM	–	–	0.006/–	Maternal
5	Exon 4	c.358G>T	p.G120C	Missense	Likely pathogenic	–	Disease causing	Damaging	0.005/–	Paternal
	Intron 18	c.2063-1g>t	Intron acceptor site AG loss	Splice-site change	Pathogenic	DM	–	–	0.006/–	Maternal
6	Exon 9	c.1007_1008insT	p.Phe336Phefs*34	Insertion	Pathogenic	–	–	–	–/–	Maternal
	Exon 12	c.1342_1343del	p.Leu448Lysfs*9	Deletion	Pathogenic	DM	–	–	–/–	Paternal
7	Intron 15	c.1677+1g>c	Intron donor site GT loss	Splice-site change	Pathogenic	–	–	–	–/–	Not determined
	Intron 18	c.2063-1g>t	Intron acceptor site AG loss	Splice-site change	Pathogenic	DM	–	–	0.006/–	Not determined
8	Exon 9	c.1043T>A	p.M348K	Missense	Likely pathogenic	–	Disease causing	Damaging	–/–	Not determined
	Exon 12	c.1342_1343del	p.Leu448Lysfs*9	Deletion	Pathogenic	DM	–	–	–/–	Not determined
9†	Exon 5	c.392C>T	p.P131A	Missense	Likely pathogenic	DM	Disease causing	Damaging	0.001/–	Paternal
	Intron 18	c.2063-1g>t	Intron acceptor site AG loss	Splice-site change	Pathogenic	DM	–	–	0.006/–	Maternal
10	Intron 7	c.888+1g>a	Intron donor site GT loss	Splice-site change	Likely pathogenic	–	–	–	0.02/–	Maternal
	Exon 10	c.1198T>C	p.S400P	Missense	Likely pathogenic	–	Disease causing	Damaging	–/–	Paternal
11	Exon 4	c.358G>A	p.G120S	Missense	Likely pathogenic	DM	Disease causing	Damaging	0.005/–	Paternal
	Intron 18	c.2063-1g>t	Intron acceptor site AG loss	Splice-site change	Pathogenic	DM	–	–	0.006/–	Maternal
12	Intron 7	c.888+1g>a	Intron donor site GT loss	Splice-site change	Likely pathogenic	–	–	–	0.02/–	Not determined
	Exon 9	c.1007_1008insT	p.Phe336Phefs*34	Insertion	Pathogenic	–	–	–	–/–	Not determined
13	Exon 9	c.1007_1008insT	p.Phe336Phefs*34	Insertion	Pathogenic	–	–	–	–/–	Not determined
	Exon 9	c.1007_1008insT	p.Phe336Phefs*34	Insertion	Pathogenic	–	–	–	–/–	Not determined
14		Not detected								
		Not detected								

DM, disease-causing mutation; *HGMD*, Human Gene Mutation Database; *SIFT*, Sorting Intolerant From Tolerant; –, no registration data.
*UCSC Genome Browser/Human Genetic Variation Database.
†Patient 9 was reported previously.[14]

were suspected to have CCD and intestinal atresia during the fetal period. One patient (#6) underwent surgery on the date of birth because of false suspicion of intestinal atresia. All patients showed persistent watery diarrhea beginning soon after birth and high fecal chloride content (>90 mmol/L). Actual stool volumes at diagnosis obtainable for 6 patients ranged from 19 to 110 g/kg/d. Among patients whose serum electrolytes were evaluated, 4 (31%), 2 (15%), and 5 (38%), respectively, showed hyponatremia (Na <130 mmol/L), hypokalemia (K <3.5 mmol/L), and hypochloremia (Cl <100 mmol/L) at diagnosis. All patients received oral NaCl. Nine (69%), 3 (23%), 1 (8%), and 4 (31%) also, respectively, received oral KCl; antidiarrheal medications such as cholestyramine, loperamide, butyrate, or polycarbophil calcium; a proton pump inhibitor (PPI); and probiotics.

Long-Term Outcome

Table IV (available at www.jpeds.com). summarizes current clinical and laboratory features including long-term outcome of the 13 patients with mutations of *SLC26A3*. Median follow-up duration was 10 years (range, 2-39). At the end of follow-up, 4 (31%) and 8 patients (62%) had no watery diarrhea and <5 stools per day, respectively; all patients had fewer stools per day than in infancy. Four (31%) and 3 patients (23%) had hypokalemia and hypochloremia, respectively, and none had hyponatremia. When they developed intercurrent respiratory infections or infectious enterocolitis, most patients had temporarily increased stool volume, serum electrolyte abnormalities, and dehydration, often requiring infusion of electrolyte solutions (data not shown). Median eGFR was 103 mL/min/1.73 m^2 (range, 79-158). Most patients had undergone only spot urine testing including sodium, potassium, and chloride concentrations, with no 24-hour urine collections. One patient had nephrocalcinosis; 3 (23%) had mild CKD. Eight (62%), 6 (46%), 1 (8%), and 3 (23%) received NaCl, KCl, antidiarrheal medication, and probiotics, respectively. Two patients (15%) received no medication. Median height, weight, and BMI were −0.5 SD, −0.6 SD, and −0.6 SD, respectively. Among 11 patients ≥6 years old, 10 (91%) received a regular education; only 1 attended special education, and only 3 had frequent soiling (27%). All 4 adult patients were employed. Three patients had a neurologic disorder (dysgraphia, tic disorder, attention deficit hyperactivity disorder, Asperger syndrome, or anxiety; 23%). Other complications and problems such as need for home parenteral nutrition, nephrocalcinosis, megacolon syndrome, enuresis, and growth hormone deficiency were seen among 3 patients (23%).

Discussion

We report 13 Japanese children with CCD and *SLC26A3* mutations with respect to clinical and genetic features. On the basis of this nationwide survey, CCD would be one of the most common congenital diarrheas and enteropathies in Japan, along with Shwachman–Diamond syndrome (24 patients in 1 report).[23] When one considers all pediatric diarrheas and enteropathies, however, CCD is very rare in Japan.

Watery diarrhea in CCD can be mistaken for urine, whereas hyperreninemia and hyperaldosteronism, leading to hyperkaluria and hypokalemia, may mimic Bartter syndrome.[6,11,24] Such early misinterpretations can delay CCD diagnosis by months or years. Close attention to fetal ultrasonographic findings characteristic of CCD is important for accurate early diagnosis.[20-22] In this study, 9 patients (69%) initially suspected to have CCD (6) or intestinal atresia (3) by fetal ultrasound scan were diagnosed neonatally with CCD. CCD diagnosis was earlier than in previous reports,[13,24,25] probably because of advances in fetal ultrasound equipment. CCD must be distinguished from intestinal atresia or other congenital abnormalities prenatally because CCD is treated medically rather than surgically.[26] A patient born in the 2000s (#6) underwent surgery upon birth because fetal ultrasound scan suggested intestinal atresia. Pediatricians, obstetricians, and pediatric surgeons should be aware of CCD as a diagnostic alternative to Bartter syndrome and intestinal atresia.[13]

A previous nationwide CCD study in Finland by Hihnala et al included long-term outcomes, but all patients were born before 1990.[8] Our study is the first nationwide report including long-term outcomes in patients with CCD born after 1990. At the most recent visit, all patients between ages 2 and 39 years had fewer stools than in infancy. In 11 patients ≥6 years old at last visit, numbers of stools had decreased to <5 daily in 8 (73%), stool characteristics were improved in 3 (27%), and soiling problems were indentified in 3 (27%). Hihnala et al reported that amount of diarrhea was reduced in only 14% of patients, and soiling remained common at all ages.[8] In our study, all patients developed normally, falling within ±2 SD for height, body weight, and BMI. Among 11 patients ≥6 years old, 10 (91%) underwent general education and 1 required special education. All 4 adult patients were employed. No patient in this study developed inflammatory bowel disease, although *SLC26A3* mutations reportedly have contributed to onset of inflammatory bowel disease in some patients.[9,27] Overall, then, long-term outcome was fairly good. We believe that early diagnosis and intervention improved outcomes compared with previous reports.[8,28]

Three patients (23%) received antidiarrheal medications, which were discontinued because of ineffectiveness. In the study by Hinahla et al, antidiarrheals also had a limited effect.[8] The only agent showing even moderate temporary effect was cholestyramine, which binds bile acids and reduces intestinal secretion, reducing diarrhea somewhat for 2-4 weeks. In children, short courses to reduce diarrhea and prevent soiling temporarily may be beneficial.[9] One patient (#2) in this study received cholestyramine during infancy, without benefit. Two patients (15%; #8 and 9) received PPI for diarrhea, but effectiveness has been controversial; instead, salt substitution should be optimized.[8,28,29] Although patients 8 and 9 achieved long-term normalization of serum

electrolytes, so did others who never received PPI; moreover, patient 8 still had soiling problems.

Frequencies of hyponatremia, hypokalemia, and hypochloremia at diagnosis resembled those in previous reports.[13,25] Nine patients (69%) received both NaCl and KCl during infancy. Previous studies reported that NaCl or KCl monotherapy might result in renal dysfunction or growth retardation, so both NaCl and KCl were needed.[8,30] Hyperreninemia and hyperaldosteronism from inadequate salt substitution can result in juxtaglomerular hyperplasia, hyalinization of glomeruli, calcium deposition, and vascular changes.[31] Oral administration of both NaCl and KCl during infancy was more common in our study than in a previous Austrian report.[24] Altogether, available studies indicate that sodium, potassium, and chloride supplementation is more important than antidiarreal therapy or PPI.[8,9,28]

As for long-term electrolyte balance, 4 (31%) and 3 patients (23%), respectively, showed hypokalemia and hypochloremia; none had hyponatremia. Five patients (38%) continued both NaCl and KCl long term, and 9 (69%) received both during infancy. Long-term persistence of hypokalemia and hypochloremia was more frequent in this study than in a previous report.[8] Normalization of serum potassium and chloride is important in preventing renal injury in CCD. Wedenoja et al examined 24-hour urinary chloride concentrations to assess long-term therapeutic response to electrolyte supplements in CCD.[7,9] In patients between 3 and 7 years old, urinary chloride should be 10-30 mmol/L, and in older children, at least 30-50. In adults, physicians additionally should monitor eGFR because of the risk of CKD.[9] In our study, only 1 patient developed nephrocalcinosis, and 3 had mild CKD. CKD clearly is associated with both delayed CCD diagnosis and inadequate salt replacement, particularly during early in life.[7] Most patients in our study had no monitoring of 24-hour urinary chloride, leading to suboptimal salt supplementation. We recommend that physicians treating patients with CCD should evaluate not only serum electrolytes and eGFR but also 24-hour urinary chloride to ensure salt replacement sufficient to prevent CKD.

The *SLC26A3* gene, previously known as down-regulated in adenoma, is located on chromosome 7q31 and consists of 21 exons. Its product is an apical transmembrane protein with 764 amino acids, including 14 probable hydrophobic membrane-spanning domains and a cytoplasmic COOH-terminal tail. The tail possesses 2 protein interaction motifs: a sulfate transporter and anti-sigma antagonist (STAS) domain including amino acids 525-720, and a post-synaptic density 95/discs large/zona occlusion–binding domain including amino acids 762-764.[11,32] Of the 101 mutations reported previously, 68 and 29 are located in the transmembrane domain and the STAS domain, respectively. Similarly, the 12 mutations among our patients involved these domains (9 transmembrane, 3 STAS), which clearly are essential to anion-exchange functions of the *SLC26A3* protein (**Figure 2**).

We confirmed that the parents who provided DNA all were carriers. Moderate evidence exists for pathogenicity of the variants.[16] We identified 6 novel mutations: 2 splice-site mutations (c.888+1 g>a and c.1677+1 g>c) in patients #7, 10, and 12, and 4 missense mutations (c.358G>T, c.1043T>A, c.1198T>C, and c.1644C>G) in patients #1, 5, 8, and 10. Splice-site mutations can disrupt gene function by causing complete transcription failure or nonsense-mediated decay of an altered transcript, and the 4 novel missense mutations either are located in critical functional domains or would lead to a missense amino acid change at the same position as another known pathogenic missense change.[11] Considering ACMG standards and guidelines,[16] these 6 novel mutations could affect synthesis or activity of the SLC26A3 protein, thus resulting in CCD.

The c.2063-1g>t mutation was found in at least 1 allele of 7 patients. This mutation was identified in all reported Korean patients, but was not observed in Finland, Poland, or Arab countries.[11,13] Accordingly, c.2063-1g>t might represent a founder mutation in East Asia, even though *SLC26A3* mutations causing Japanese CCD are more diverse than those reported from Korea. Of 101 previously known mutations, 3 (c.559G>T, c.951_953del, and c.2024_2026dup) account for 47%-94% of mutations in Finland, Poland, and Saudi Arabia[10]; distributions of these mutations have been explained as founder effects. According to HGVD, frequencies of c.559G>T, c.951_953del, and c.2024_2026dup are nil. Thus, the mutation spectrum of *SLC26A3* in CCD shows ethnic variation.

Although 12 types of pathogenic variants of *SLC26A3* were identified in 13 Japanese patients, correlation between genotype and phenotype in CCD remains limited. Previous reports have stated that patients with the same genotype showed different responses to diarrhea-modulating agents such as butyrate, with benefit for Italian but not Finnish patients.[33-35] Study of additional patients may clarify genotype–phenotype relationships. Some limitations in this study could be addressed in future research. First, surveys were limited to pediatric centers, so little new knowledge was gained about CCD in adults. Second, compared with studies from Europe,[3,11] our patient numbers were relatively small because of lower prevalence of CCD. Additional systematic identification of cases in other East Asian countries will be important.

In conclusion, we examined clinical features, molecular genetics, and long-term outcomes in Japanese patients with CCD. Early diagnosis by fetal ultrasound scan and fecal chloride determinations, differentiating CCD from congenital intestinal atresia or Bartter syndrome, and early initiation of long-term treatment with both NaCl and KCl may be important to improve outcomes. We identified 12 abnormalities of *SLC26A3*, including 6 novel mutations, among 13 patients. Another patient had no mutation of this gene. The *SLC26A3* mutation spectrum in CCD appears to vary between ethnic groups; c.2063-1g>t might represent a founder mutation in East Asia. ■

We thank all participating patients and their families for collaborating in data collection. We also thank all physicians and surgeons who participated in the nationwide survey for data collection and Dr Takao Togawa at Nagoya City University Graduate School of Medical Sciences for insightful advice on the genetic analysis.

Submitted for publication Apr 26, 2019; last revision received Jun 26, 2019; accepted Jul 12, 2019.

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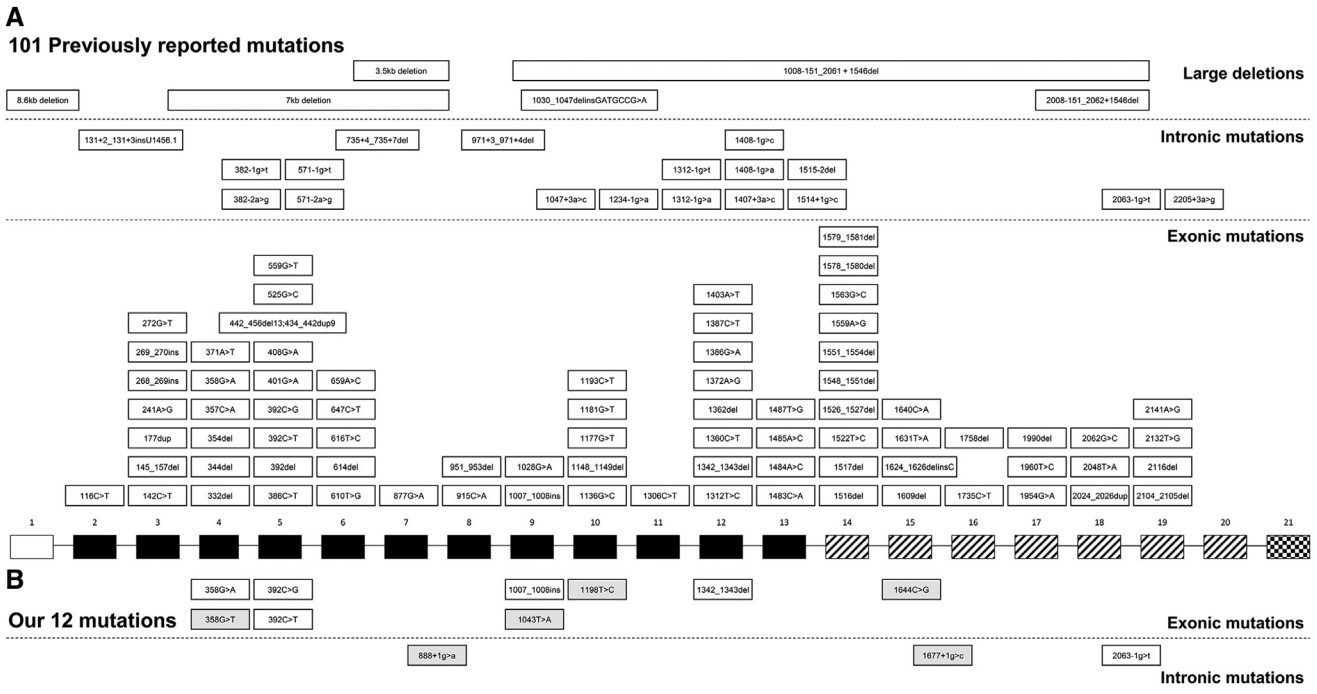

Figure 2. Location of the mutations in *SLC26A3* observed in congenital chloride diarrhea. The translation initiation codon and the termination codon are located in exons 2 and 21, respectively. The probable hydrophobic membrane-spanning domains, the STAS domain, and the post-synaptic density 95/discs large/zona occlusion interaction motif, respectively, are depicted as *black*, *diagonally striped*, and *checkered boxes*. **A,** The 101 pathogenic mutations in patients with congenital chloride diarrhea registered in the Human Gene Mutation Database and in previous reports. These mutations are divided into 3 types: large deletions, intronic mutations, and exonic mutations. **B,** The 12 mutations detected in this study. The 6 novel mutations are shaded in *light gray*.

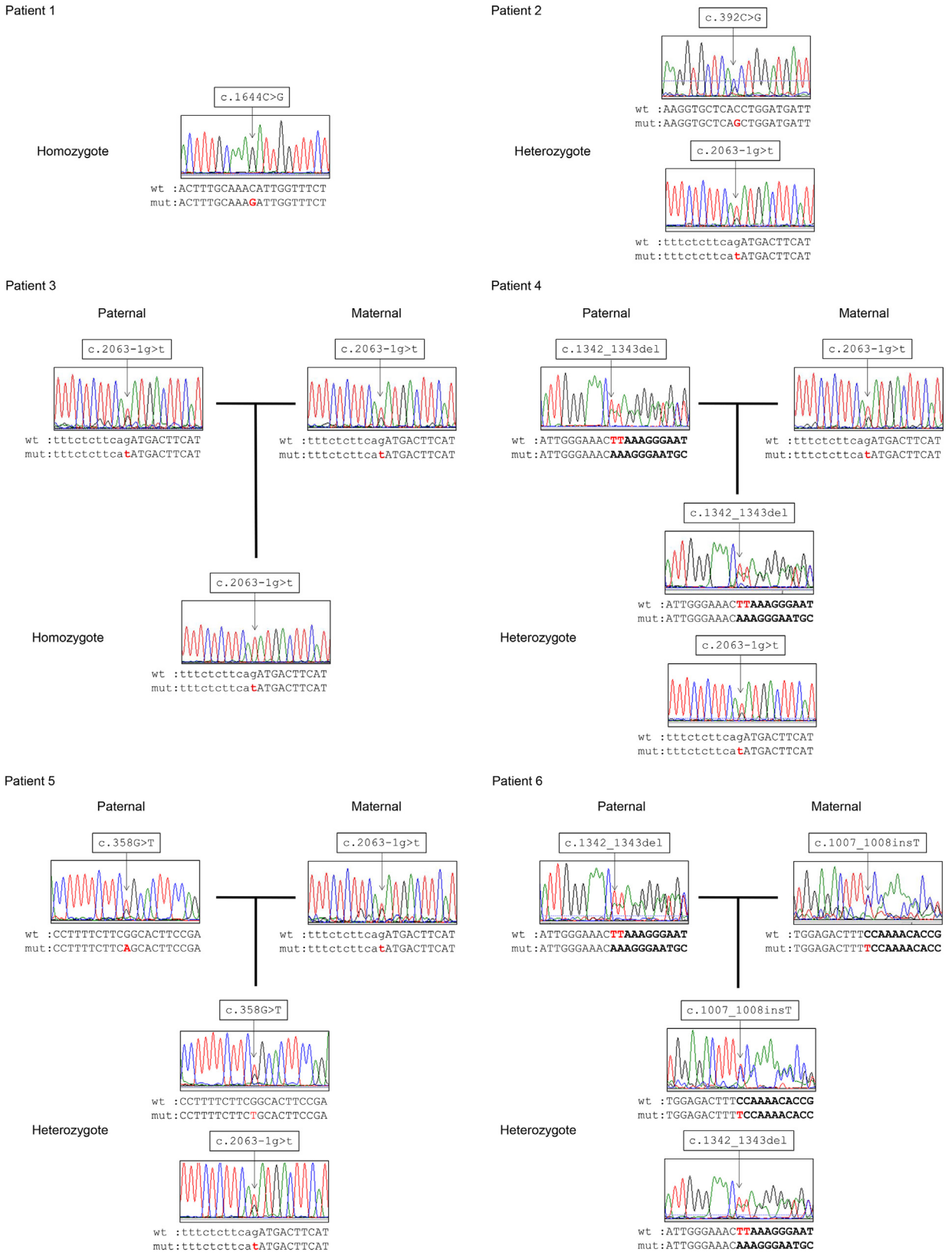

Figure 3. Sanger sequencing showing *SLC26A3* mutations observed in the patients and their parents. Mutation sites are indicated by *arrows* in each diagram. Patients 1 and 3 had homozygous aberrations; patients 2, 4-8, 10, 11, and 12 had compound heterozygous abnormalities. DNAs from the parents of patients 3-6, 10, and 11 were available, and we confirmed that they were carriers. *mu*, mutation; *wt*, wild type. *(Continues)*

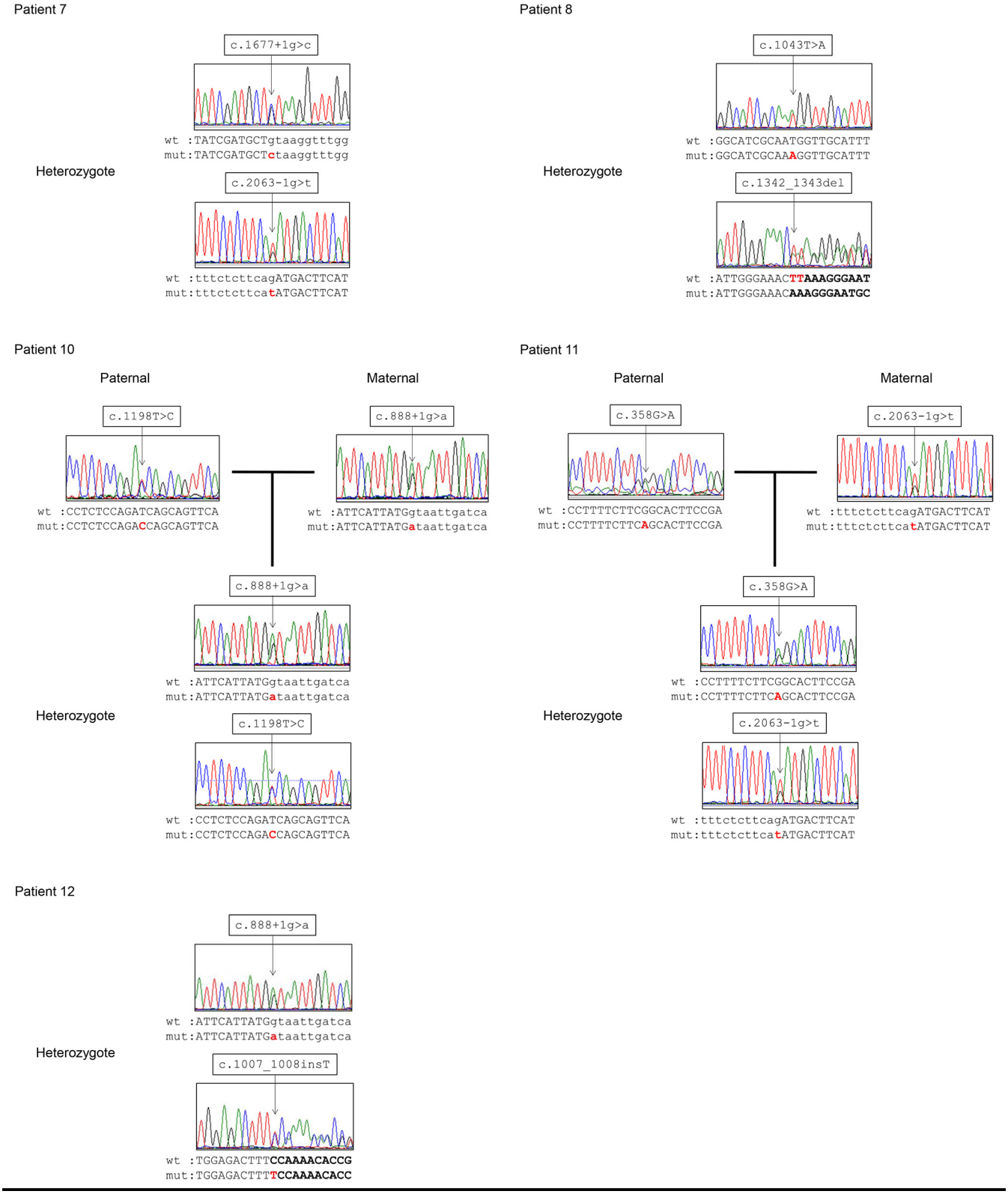

Figure 3. (Continued)

Table I. Primers used for polymerase chain reaction to detect *SLC26A3* mutations

Exons	Sense primer 5′ → 3′	Antisense primer 5′ → 3′	Expected size, bp
1	AAGGTCAAGACCACATTGCAG	CAAGGAACAGAAAACCAGTTTGA	297
2	TTCAGTTCGGAGCTCACTCA	TCAAAAATGTATTCCTAATGCCAAT	344
3	TACCAATTTCCCACCGACAG	CACATTCAGAGGAAGAATTTTATTG	272
4	CACAAAGATTTATACCTATGCTTTCTT	AAAACAATGTGAGCATTAATCAGC	344
5	ACCACGATTCACCCACTGTT	GCCATGAGGGAGAGACAAAC	340
6	GCAGGGACATTTGCTTCTTC	CATGGCAAAGGCATTAAAGG	396
7	TCAGTCTTTTTGCTTCCATGTT	AGCTATGACAGAGTCTGAAAATGA	290
8	GCCTGGGCTGGAGTTCTTAC	ATGCCATCATCGAAGACACA	229
9	CCAAAGTGAGACACAACTCAGC	GCCCAGTGAGACCCAGTGTA	295
10	TGGCAATCAGGTAAGTAAACAAA	CCCCTGGCTTGAATTTCCTA	300
11	TCTTTGCTGCAAGCACTGTC	AACCGGGGTCCTTGATTTAC	229
12	GAGGCCCTTTAAAGCACACA	TGCTATTGTGATTTATCTCTCTTTCA	244
13	AGTGTATGTGTTGCTTTCTTTTCA	GAAATGCACACCTATCAACCAA	247
14	GAAAAGTGAGCCTGCTTTGG	GCAAGTCAAAGAAAAACATGAATC	203
15	TTGGAAGAACCAACATCTATAAGAA	CAGAACAATGACATTCCCAGAA	297
16	CCCAATCCCTCAAATCACTC	TGCATAGAAATGTGGTCAAGG	222
17	CACCTAGTCCAATCCCATCG	TCATTGATTGGTTGTTTCCA	398
18	AAAAGGAATATATCACCCGTAAGGT	GGCAGTGAGCCTGAAGTGAT	211
19	CAAATGTCAATGAAACGCACA	GGCTGCTTATGCAATTGTGA	298
20	TCCCAGTCAGGTATCAGCTCT	TCTGCCACATGCAGGAAGT	287
21	TGGTAAATTCTAGCTCCTAAGATGTG	TTCTGTCAAAAATACTCTTCGTACAAT	218

Table III. Patient characteristics during the perinatal period and infancy

Patients	Decade of birth	Sex	Gestational week at birth	Birth weight, g	Polyhydramnios	Dilated fetal bowel loops	Fetal diagnosis	Age at diagnosis	Clinical and laboratory findings at diagnosis: Watery diarrhea, g/kg/d*	Abdominal distention	Fecal chloride content, mmol/L	Serum Na, mmol/L	Serum K, mmol/L	Serum Cl, mmol/L	Treatment during infancy
1	1970s	F	38	2585	+	NA†	–	1 mo	+	+	>90‡	**127**	4.1	**70**	NaCl, KCl
2	1970s	F	36	2985	+	NA†	–	2 mo	+	+	116	**118**	6.7	**80**	NaCl, KCl, cholestyramine
3	1970s	F	33	2640	+	NA†	–	8 mo	+	+	117	**127**	**1.9**	**53**	NaCl, KCl, herbal medicine
4	1990s	M	36	2755	+	+	CCD	0 d	+ (73)	+	149	138	4.8	112	NaCl, KCl, loperamide, probiotics, elemental diet
5	2000s	F	34	2156	+	+	CCD	1 d	+	+	140	139	3.8	102	NaCl, KCl, butyrate, polycarbophil calcium, diuretics
6	2000s	M	32	2234	+	+	CIA	0 d	+	+	142	139	5.7	108	NaCl, probiotics
7	2000s	F	37	3156	+	+	CCD	0 d	+ (63)	+	102	137	3.5	**93**	NaCl, KCl
8	2010s	M	28	970	NA§	NA§	–	3 mo	+	–	120	136	4.3	103	NaCl, $NaHCO_3$
9	2010s	M	37	2910	+	+	CIA	0 d	+ (110)	–	149	142	5.2	111	NaCl, PPI, probiotics
10	2010s	M	37	2396	+	+	CCD	3 d	+ (19)	–	144	137	4.1	101	NaCl, KCl
11	2010s	M	37	3092	+	+	CCD	0 d	+	–	147	**120**	NA	**90**	NaCl
12	2010s	F	36	2398	+	+	CCD	1 d	+ (97)	+	146	135	5.3	106	NaCl, KCl, probiotics
13	2010s	M	36	2748	+	+	CIA	20 d	+ (94)	+	134	145	**3.3**	111	NaCl, KCl, diuretics, herbal medicine
Median (range)			36 (28-38)	2640 (970-3156)				1 d (0 d-8 mo)			141 (102-149)	137 (118-145)	4.2 (1.9-6.7)	102 (53-112)	

CIA, congenital intestinal atresia; *Cl*, chloride; *F*, female; *K*, potassium; *M*, male; *Na*, sodium; *NA*, data not available; *NaCl*, sodium chloride; *$NaHCO_3$*, sodium hydrogen carbonate.

*Actual stool volumes at diagnosis.

†Patients 1, 2, and 3 showed no information concerning dilated fetal bowel loops because fetal ultrasound scan was performed in the 1970s.

‡Exact fecal chloride content in patient 1 was unknown, although it exceeded 90 mmol/L.

§Patient 8 did not undergo fetal ultrasound scan because he was born at an extremely low birth weight following premature rupture of membranes. Abnormal values of serum electrolytes, hyponatremia (Na<130 mmol/L), hypokalemia (K<3.5 mmol/L), and hypochloremia (Cl<100 mmol/L) are shown in bold.

Table IV. Most recent clinical and laboratory findings including long-term outcome

Patients	Duration of follow-up, y	Fecal characteristics	Number of stools per day	Serum Na, mmol/L	Serum K, mmol/L	Serum Cl, mmol/L	eGFR, mL/min/1.73 m^2	Treatment	Height, SD	BW, SD	BMI, SD	School/ employment	Soiling problems	Neurologic disorders (onset ages of years)	Other complications and problems (onset ages of years)
1	39	Watery	5-9	139	**2.9**	103	106	–	−0.4	−0.7	−0.6	General education, employed	–	–	–
2	39	Watery	1-4	135	**2.9**	**92**	**79**	KCl	−0.6	−0.4	−0.2	General education, employed	–	–	–
3	39	Watery	1-4	142	**2.9**	102	158	NaCl, KCl, herbal medicine	1.4	−0.4	−0.9	General education, employed	–	–	HPN (33), Nephrocalcinosis (32), megacolon syndrome (33)
4	22	Watery	1-4	141	4.7	101	**80**	–	−0.5	−0.2	0.0	General education, employed	–	–	–
5	16	Watery	5-9	144	**3.0**	102	100	NaCl, KCl	0.3	−0.7	−0.9	General education	+	–	–
6	13	Mushy	1-4	135	3.8	**99**	110	NaCl	−0.1	−0.1	0.2	General education	+	Tic disorder (9)	Enuresis (9)
7	10	Loose	1-4	140	3.9	103	93	NaCl, KCl	−0.8	−0.1	0.7	General education	–	Dysgraphia (9)	–
8	9	Loose	1-4	140	3.5	105	106	Polycarbophil calcium, PPI, probiotics	−0.7	−1.1	−1.5	Special education	+	ADHD (3), Asperger syndrome (8), anxiety (8)	Growth hormone deficiency (3)
9	7	Watery	1-4	139	4.0	103	117	PPI, probiotics, herbal medicine	0.1	−0.5	−0.7	General education	–	–	–
10	6	Watery	5-9	138	3.7	100	108	NaCl, KCl	−0.4	−1.2	−2	General education	–	–	–
11	6	Watery	1-4	140	4.4	107	**81**	NaCl	−0.8	−0.8	−0.6	General education	–	–	–
12	2	Mushy	5-9	137	4.7	103	95	NaCl, probiotics	−1.4	−0.6	0.5	Preschool age	NA	–	–
13	2	Watery	5-9	139	3.5	**96**	103	NaCl, KCl, diuretics	−1.7	−1.6	−0.6	Preschool age	NA	–	–
Median (range)	10 (2-39)		1-4	139 (135-144)	3.7 (2.9-4.7)	102 (92-107)	103 (79-158)		−0.5 (−1.7 to 1.4)	−0.6 (−1.6 to −0.1)	−0.6 (−2 to 0.7)				

ADHD, attention deficit hyperactivity disorder; *BW*, body weight; *HPN*, home parenteral nutrition.
Abnormal values of serum electrolytes, hyponatremia (Na <130 mmol/L), hypokalemia (K <3.5 mmol/L) and hypochloremia (Cl <100 mmol/L), and eGFR (<90 mL/min/1.73m^2) are shown in bold.