Phenotype and Genotype of a Cohort of Chinese Children with Early-Onset Protein-Losing Enteropathy

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Objectives To examine the phenotypes and perform next-generation sequencing in children with early-onset protein-losing enteropathy.

Study design We performed a retrospective review of 27 children with early-onset protein-losing enteropathy. Patients were characterized on clinical, immunologic, and systemic involvements. Targeted gene panel sequencing and whole-exome sequencing were performed in 9 patients.

Results In 27 patients (55.6% male), median age of disease onset was 173 days, and 59.3% had onset of disease before 1 year of age. Initial gastrointestinal symptoms included diarrhea (74.1%), vomiting (33.3%), and abdominal distention (48.1%). All patients had hypoalbuminemia, with an average serum albumin concentration of 20.2 ± 5.4 g/L. Hypogammaglobulinemia was identified in 72% of the patients. Upper endoscopy showed typical presentation of intestinal lymphangiectasia (n = 13). Patients frequently received intravenous albumin and immunoglobulin infusions as well as parenteral nutrition. Next-generation sequencing in 9 patients with available DNA showed 1 patient had compound heterozygous *CCBE1* mutations and 2 had novel homozygous *DGAT1* mutations. Monogenic diseases were identified in 3 of 9 patients who underwent genetic sequencing. Three subjects (11.1%) died, of whom 2 had homozygous *DGAT1* mutations. No significant correlation was found between age of symptom onset, serum albumin, serum IgG, lymphocyte count, $CD4^+$ cells, and mortality.

Conclusions Monogenic diseases may be observed in children with early-onset protein-losing enteropathy, and genetic evaluation with next-generation sequencing should be considered. *(J Pediatr 2019;208:38-42)*.

Protein-losing enteropathy (PLE) is associated with various gastrointestinal conditions, including Crohn's disease, celiac disease, and intestinal lymphangiectasia.[1] Patients generally exhibit hypoproteinemia, edema, and immune dysregulation.[2] Syndromic causes of PLE include Hennekam syndrome with *CCBE1* or *FAT4* mutations,[3] generalized lymphatic dysplasia caused by *PIEZO1* mutations,[4] and lymphedema–distichiasis syndrome caused by *FOXC2* mutations.[5,6]

There are reports of PLE in the setting of nonsyndromic primary intestinal lymphangiectasia.[7,8] These patients often have disease onset early in life and are defined as congenital enteropathy due to monogenic disorders.[9] *CD55* mutation has been reported to cause autosomal-recessive CHAPLE (complement hyperactivation, angiopathic thrombosis, and protein-losing enteropathy) syndrome, with hyperactivation of complement, angiopathic thrombosis, and early-onset PLE.[2] Mutations in acyl-CoA:diacylglycerol acyltransferase (*DGAT*) *1* are reported to cause congenital PLE owing to aberrant lipid metabolism.[10] Here, we report a large cohort of patients with early-onset PLE[11] in whom we also conducted next-generation sequencing to examine genetic causes in a subset.

Methods

A retrospective study was conducted on patients with hypoproteinemia due to PLE admitted to Children's Hospital of Fudan University from 2011 to 2017. Patients with hypoalbuminemia, defined as serum albumin lower than normal range (reference value 33-55 g/L), with/without edema were first identified. We then excluded patients with other causes of albumin loss (eg, proteinuria), inadequate synthesis (eg, liver disorders), and inadequate supply (eg, protein malnutrition). Patients with secondary PLE, including Fontan procedure, and other known conditions causing gastrointestinal protein loss (including inflammatory bowel disease or celiac disease) also were excluded.

Clinical indices and results of diagnostic studies were retrieved from electronic medical records. All patients underwent complete blood count, chemistry, and immunoglobulins (IgA, IgG, IgM). The results of gastrointestinal endoscopy

CT	Computed tomography
DGAT	Acyl-CoA:diacylglycerol acyltransferase
PLE	Protein-losing enteropathy
TGPS	Targeted gene panel sequencing

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The authors declare no conflicts of interest.

This study was presented as poster in The Translational Science of Rare Disease, From Rare to Care III on April 12, 2018.

0022-3476/$ - see front matter.
https://doi.org/10.1016/j.jpeds.2018.12.003

and histologic analysis by hematoxylin and eosin staining were reviewed, as were reports of imaging studies (abdominal ultrasound scan, enhanced computed tomography [CT] of the abdomen, and echocardiogram). Weight-for-age z score and height-for-age z score on initial diagnosis were obtained by WHO Anthro (version 3.2.2, January 2011; World Health Organization, Geneva, Switzerland).

We performed targeted gene panel sequencing (TGPS) in 7 patients and whole-exome sequencing in 2 patients with available DNA. TGPS covered pathogenic genes for 3520 genetic diseases previously reported in Online Mendelian Inheritance in Man. Genomic DNA was extracted from peripheral whole blood of patients and their parents. Whole-exome sequencing resulted in an average 100× coverage using the HiSeq X platform (Illumina, San Diego, California). Sequence read alignments were completed using NextGene V2.3.4 (SoftGenetics, State College, Pennsylvania) against the human reference genome GRCh37/hg19. Variants were filtered with public database after quality control, including Human Gene Mutation Database Professional, 1000 Genome, dbSNP, Exome Aggregation Consortium, and in-house database. Sanger sequencing was performed with Biosystems 3500 DNA Analyzer (Thermo Fisher Scientific, Waltham, Massachusetts) and analyzed by Mutation Surveyor V4.0.9 (SoftGenetics) to confirm the causal mutations. Bioinformatics pipeline of whole-exome sequencing was adopted as previously reported.[12] In silico prediction tools, including Mutation Taster[13] and Human Splicing Finder,[14] MaxEntScan,[15] were applied for all identified novel mutations.

A recent study concluded that CD55 deficiency leads to early-onset PLE.[2] This gene was not included in the targeted gene panel. Thus, DNA Sanger sequencing of the gene coding CD55 was performed. Respective primer sequences are designed according to previously reported[2] and shown in **Table I** (available at www.jpeds.com).

Statistical Analyses

Data were analyzed using SPSS 21.0 for Windows (IBM Corp, Armonk, New York). Continuous data are presented as mean and SD or IQR. Categorical variables are reported as counts and percentages. Spearman correlation analysis was applied.

Ethical Considerations

This study was approved by the Ethical Committee of Children's Hospital, Fudan University. Informed consents for participation for genetic sequencing were obtained from parents or legal guardians of the patients.

Results

Between 2011 and 2017, we identified 3161 subjects with low serum albumin and excluded 2853 subjects with hypoalbuminemia with albumin loss due to nephrotic diseases, 199 patients with inadequate synthesis due to liver diseases (n = 146) and malignancy (n = 53), and 82 patients with secondary PLE, leaving 27 subjects with primary PLE. Among them, 55.6% were male. Median parent-reported age of onset of disease was 173 days (IQR 60-931). Among all subjects, 59.3% (16/27) patients had onset of disease within 1 year of age. Consanguineous marriage was denied in all cases after detailed family history taking. Patients were born originally from 14 different provinces in China. Of our patients, 26 were of Han Chinese origin, and 1 patient was Chinese Uyghur. None of the patients had a positive family history of PLE.

Table II. Summary of phenotypes, diagnostic findings, and treatment in patients with early-onset PLE

Variables	n (%)
Male sex	15/27 (55.6)
Onset within 1 y of age	16/27 (59.3)
Presenting symptoms	
Diarrhea	20/27 (74.1)
Vomiting	9/27 (33.3)
Abdominal distension	13/27 (48.1)
Edema	21/27 (67.7)
Infection	10/27 (37.0)
Laboratory tests	
Serum albumin, g/L	20.2 ± 5.4
Intestinal lymphangiectasia	13/25 (52.0)
Hypogammaglobulinemia	18/25 (72.0)
Hypertriglyceridemia	1/27 (3.7)
Treatment	
Albumin transfusion	25/27 (92.6)
Immunoglobulin transfusion	17/27 (63.0)
MCT-based formula	15/27 (55.6)
Parenteral nutrition	6/27 (22.2)

MCT, medium-chain triglycerides.

On admission, median weight-for-age z score was 0.33 (IQR −0.90, 0.78) and median height-for-age z score was −0.27 (IQR −2.15, 0.60). Fourteen percent of patients had height-for-age z scores of less than −2, and 7.4% had weight-for-age z scores of less than −2. Presenting symptoms included a wide range of gastrointestinal symptoms (**Table II**). All patients had at least 1 previous hospitalization in local hospitals. **Table III** (available at www.jpeds.com) shows details regarding individual data.

Laboratory tests on presentation also are noted in **Table II**. All 27 patients had hypoalbuminemia, 23 (85.2%) had hypogammaglobulinemia, 6 (22.2%) had elevated alanine aminotransferase (reference value 0-40 IU/L), 7 (25.9%) had elevated aspartate aminotransferase (reference value 15-60 IU/L), 3 (11.1%) had lymphopenia (reference value 4-8.4 × 10^9/L), and 1 had hypertriglyceridemia of 2.66 mmol/L (reference value 0.56-1.7 mmol/L).

There was a positive correlation between serum albumin and IgG level (Spearman r = 0.48, P = .014). Common supportive therapies (**Table II**) included intravenous albumin, gamma globulin, and parenteral nutrition.

Immunologic investigations showed median (IQR) IgA 0.33 (0.12-0.54) g/L, IgG 2.85 (0.63-4.47) g/L, and absolute $CD4^+$ 0.7 × 10^9/L (0.2-1.2) × 10^9/L. Compared with age-specific reference ranges,[16,17] 12 (48.0%) patients had

low level of serum IgA, 18 (72.0%) had low level of serum IgG, and 12 (54.5%) had low numbers of $CD4^+$ T cells.

Upper gastrointestinal endoscopy was performed in 25 patients. Scattered white dots (**Figure**, A) were found in the descending part of duodenum in 13 (52.0%) patients. Pathologic investigations showed dilated lymphatics in 17 (77.3%) patients. Echocardiogram was performed in 16 of 27 patients, which showed mild tricuspid regurgitation (n = 1), atrial septal defect (n = 2), patent foramen ovale (n = 3), pericardial effusions (n = 1), and ventricular septal defect (n = 1). Of all, 20 of 27 (74.1%) had abdominal ultrasound scans and 23 of 27 (85.2%) had enhanced CT of the abdomen. Ascites were found in 12 (57.1%) patients. Enhanced CT of the abdomen among patients revealed intestinal wall enhancement (**Figure**, B), edematous mesentery, and ascites. For individual diagnostic results, see **Table III**.

We identified compound heterozygous *CCBE1* c.521C>T p.C174Y/c.271C>T p.D91N mutations in case 1, homozygous *DGAT1* c.895-1G>A in case 24, and homozygous *DGAT1* c.1249-6T>G mutations in case 27. In the remaining 6 patients undergoing genetic sequencing, we did not identify any known disease-causing mutations. Results of next-generation sequencing and in silico analysis are shown in **Table IV**. The scores of authentic and mutant slice sites predicted by Human Splicing Finder and MaxEntScan are listed in **Table V** (available at www.jpeds.com). No pathogenic variants were identified in CD55 among all 9 patients undergoing Sanger sequencing.

Most patients were stabilized and discharged. Three patients died (11.1%), 2 of whom had homozygous *DGAT1* mutations. There was no significant correlation between age of symptom onset, serum albumin, serum IgG, lymphocyte count, $CD4^+$ cells, and mortality.

Discussion

Of the 9 subjects tested, we identified 1 patient with compound heterozygous *CCBE1* and 2 with novel *DGAT1* mutations. Two of the 3 patients with DGAT1 mutations died, suggesting that this genotype is associated with an especially severe phenotype.

Ozen et al reported that 8 of 10 patients with early-onset PLE had onset of disease before 2 years of age.[2] In our study, patients generally suffered from hypoalbuminemia and lymphopenia, and they presented with gastrointestinal symptoms including watery diarrhea and abdominal pain. Typical manifestations of diarrea, hypoalbuminemia, and recurrent infections are consistent with previous reports regarding early-onset PLE.[2,10,11] Most patients had intestinal lymphangiectasia detected by endoscopy. Cardiac defects, including atrial septal defects and patent foramen ovum, also were common among patients, which was consistent with other studies.[2,4,18,19]

Limited previous data link PLE with pathogenic *DGAT1* mutations.[8,11,20,21] We identified 2 patients with novel

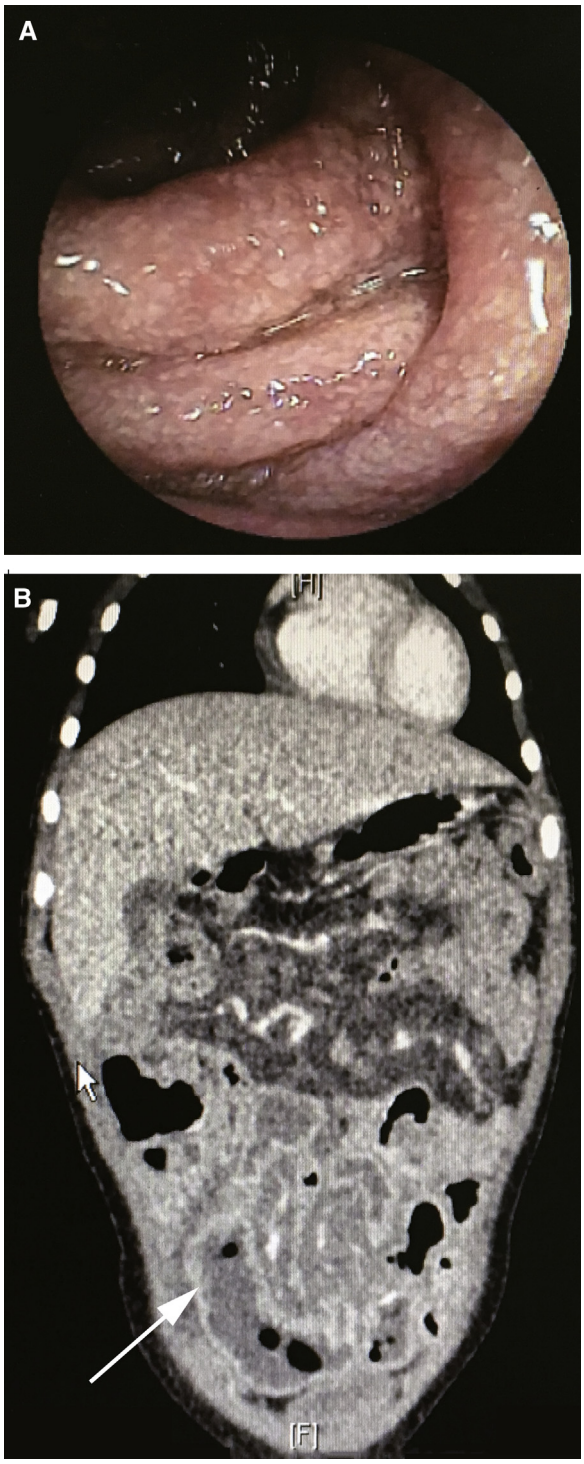

Figure. **A,** Upper endoscopy findings showed typical scattered white dots in descending part of duodenum in case 8. **B,** Abdominal CT indicates diffuse intestinal wall enhancement in case 10.

DGAT1 mutations (homozygous *DGAT1* c.895-1G>A, and homozygous *DGAT1* c.1249-6T>G). The *DGAT1* c.895-1G>A variant is a canonical splice site variant and is classified as a PVS1-null variant according to American

Table IV. Summary of next-generation sequencing among 9 patients with early-onset PLE

Case	Gene	Reference sequence	Mutation		Mutation taster	Human splicing Finder	Sequencing method
1	*CCBE1*	NM_133459	c.521C>T	p.C174Y*	D	–	Panel
			c.271C>T	p.D91N	D	–	
3	Negative						Panel
6	Negative						Panel
10	Negative						Panel
13	Negative						Panel
14	Negative						Panel
17	Negative						Panel
24	*DGAT1*	NM_012079	c.895-1G>A (Homozygous)	N/A	–	Splicing	WES
27	*DGAT1*	NM_012079	c.1249-6T>G (Homozygous)	N/A	–	Splicing	WES

D, disease-causing; *N/A*, not available; *WES*, whole-exome sequencing.
*Mutation reported to be pathogenic in the Human Gene Mutation Database.

College of Medical Genetics and Genomics guidelines.[22] In silico prediction programs such as MaxEntScan and Human Splicing Finder showed great effect on acceptor site for the mutation *DGAT1* c.1249-6T>G, which will affect splicing. van Rijn et al recently reported 10 patients from 6 families with *DGAT1* mutations and PLE.[10] All 10 presented with early-onset severe diarrhea, PLE, and malnutrition. The enzymes DGAT1 and DGAT2 catalyze triglyceride biosynthesis, and compared with other monogenic causes of PLE, homozygous complete loss-of-function mutations in *DGAT1* lead to severe congenital diarrhea syndrome, associated with hypertriglyceridemia, which was the feature of human DGAT1 deficiency.[21]

Next-generation sequencing of 9 patients showed causative *CCBE1* mutations (c.521C>T/c.271C>T) in 1 case. The c.521C>T mutation has been reported previously by Alders et al, where *CCBE1* mutations were found in 25% of patients with lymphedema and lymphangiectasia.[18] Pathogenic *CCBE1* mutation was first described in 2009,[7] and patients generally presented with lymphedema, lymphangiectasia, intellectual disability, and dysmorphic facial features. Functional analysis in zebrafish showed that *CCBE1* mutants lead to absence of lymphangioblasts and lymphatic vessels.[23]

Ozen et al described patients with *CD55* mutations presenting with early-onset gastrointestinal complaints, including chronic diarrhea and abdominal pain and PLE.[2] Endoscopic and pathologic investigations showed pronounced intestinal lymphangiectasia. Our patients had similar phenotypes. Because *CD55* is not included in the TGPS, we performed Sanger sequencing of this gene. However, Sanger sequencing did not identify any pathogenic mutations in *CD55*.

Supportive therapy for patients with PLE commonly includes intravenous albumin and/or gamma globulin, micronutrient supplementation, enteral supplementation with medium chain triglycerides, and parenteral nutrition as needed. Kurolap et al recently reported that patients with PLE due to CD55 deficiency responded to a 100-day course of eculizumab, a terminal complement inhibitor, with increased serum albumin concentrations and fewer gastrointestinal symptoms.[24] Patients with CD55 deficiency had in vitro complement overactivation, and eculizumab may be beneficial for these patients.[25]

There were several limitations of this study, including its retrospective design, and limited genetic evaluation (only 9 of 27 patients underwent sequencing). Nonetheless, our finding of pathologic monogenic mutations in 3 of 9 patients tested, as well as the greater mortality rates in those with *DGAT1* mutations, support the wider application of genetic sequencing in these patients. Prospective, multicenter studies are needed to evaluate rare diseases such as early-onset chronic diarrhea syndromes and PLE. ■

We thank all the patients and families who participated in the study.

Submitted for publication Jul 17, 2018; last revision received Nov 20, 2018; accepted Dec 4, 2018.

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Table I. Primer sequence of CD55

Exon	Forward primer sequence	Reverse primer sequence
Exon 1	CTACTCCACCCGTCTTGTTTGT	TTTGGGGGTTAAGGATACAGTC
Exon 2	CAGGTGTGGCATTTCAAGG	ACCCTGGGGTTTAGTAACGC
Exon 3	AAGTACTAAATATGCGCAAAGCAG	ATGGTCCTATCAAGAAACATCC
Exon 4	GTTACCTTCTTTGTGTGTATGCC	GCTGTGAATACCAGTCATGAAAC
Exon 5	AACCTGGAGAATTTGAGGAAAG	TGTGCTAATATTCTTAAGGGGC
Exon 6	GCATTTATAAGCATCTCTTGTTGG	TCATTGAATGTCTGCAACCC
Exon 7	CTAGGTGTTTGTGGGGAGAGAG	TCTGGTGGGTTTCTGAAGAGTT
Exon 8	TTTACGCAGAGTCCTTCAGC	CCATTTAATCCTGCAATCTTGG
Exon 9	TGGAAATTTGAGTTGCTTTCG	TCTCCCAGGAATATGGATTG
Exon 10	GCACCCCAAATTAACTGATTC	ATGTGATTCCAGGACTGCC

Table III. Phenotypic characteristic, diagnostic findings, and treatment in patients with early-onset PLE

Case	Onset, mo	Sex	Serum albumin, g/L	Edema	Diarrhea	Vomiting	Intestinal lymphangiectasia	Echocardiogram	Ascites	Chylous	Abdominal ultrasound scan	Abdominal CT/MRI	Albumin transfusion	IVIG	Pepti-Junior	MCT	TPN
1	1	F	19.9	+	+			PFO			N	N	+	+	+		
2	2	F	18	+	+	+	+	NA	+		Mesenteric mass	Soft-tissue mass, mesenteric mass, multiple low density	+	+	+		+
3	30	F	26.6		+		+	NA			Nephromegaly	Nephromegaly, hydronephrosis	+				+
4	40	F	24.1	+			+	N			NA	NA	+			+	
5	55	M	17.2	+				N	+		N	N	+	+		+	
6	1	F	14.4	+	+		+	PFO			NA	Multiple plaque calcification, MLNs	+	+	+		
7	5	F	14		+	+	+	NA			N	Intensified retroperitoneum	+	+			
8	11	M	13.4	+			+	N	+		Funicular hydrocele	Thickened and edematous mesentery	+	+		+	
9	3	M	15.3		+	+	+	N			N	NA	+		+		
10	0	F	23.8	+			+	N	+		N	Thickened small intestinal wall, MLNs, low-density mesentery	+	+		+	
11	4	M	16.8		+		+	ASD			Hepatomegaly	Intensified intestinal wall, multiple LN	+	+	+		
12	86	M	16.8	+			+	TR	+		Hepatomegaly	Hepatomegaly	+			+	
13	4	M	22.4	+	+		+	N	+	+	Hydrocele	Intensified intestinal wall and mesentery	+	+	+		
14	18	M	28.5		+	+	+	PFO			N	Intensified intestinal wall and mesentery	+	+	+		
15	26	M	19.2	+	+	+	+	PE	+		N	Ascites (mass), hydrothorax	+		+		
16	7	F	15.9	+	+		+	NA			NA	Gluteal, groin, and lower abdominal thickened fat shadow	+	+	+		
17	44	M	25.7	+	+	+	+	N	+	+	Abdominal mass	Mesenteric low density, MLNs, intensified intestinal wall	+	+		+	
18	44	M	18	+	+	+	+	ASD, VSD, TR			Retroperitoneal mass, hepatomegaly	Retroperitoneal and mesenteric abnormal signal	+		+		+
19	4	M	20.1		+		+	NA			N	NA	+	+	+		
20	5	M	14.7	+	+	+	+	NA	+		N	Mesenteric edema	+	+	+		+
21	148	F	23.4	+	+			N			NA	N				+	
22	18	F	23.2					NA	+	+	Focal hepatic lesions	Thickened small intestinal wall, splenomegaly	+	+			
23	0	M	34	+	+			NA	+		N	Ascites					
24	0	F	24.4		+	+	+	ASD			NA	Intensified small intestine	+	+			+
25	0	M	14.1				+	NA			NA	N	+		+		
26	30	M	13.5	+	+			NA	+		N	Enlarged MLNs	+	+	+		
27	30	F	27.6	+				N			NA	N	+	+	+		+

ASD, atrial septal defect; *F*, female; *IVIG*, immune globulin; *M*, male; *MCT*, medium chain triglyceride; *MLN*, mesenteric lymph nodes; *MRI*, magnetic resonance imaging; *N*, normal; *NA*, not available; *PE*, pericardial effusions; *PFO*, patent foramen ovale; *TPN*, total parenteral nutrition; *TR*, tricuspid regurgitation; *VSD*, ventricular septal defect.

Table V. The predicted scores of the authentic and mutant splice sites

Case	Mutation	MaxEnt scores (0-12)		Human splicing finder scores (0-100)	
		A	M	A	M
24	*DGAT1* c.895-1G>A	8.77	0	89.12	60.18
27	*DGAT1* c.1249-6T>G	0	8.55	62.32	91.26

A, the scores of authentic splice sites; *M*, the scores of mutant splice sites.