의학석사 학위논문

Clinical Characteristics of Chronic Enteropathy Associated with *SLCO2A1* gene (CEAS) in Children and Adolescents

소아청소년기에서 *SLCO2A1* gene 연관 만성 장병증 (CEAS) 의 임상 양상

2022년 2월

서울대학교 대학원

임상의과학과 전공

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지도 교수 고재성

이 논문을 의학석사 학위논문으로 제출함

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임상의과학과 전공

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2022년 1월

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Abstract

Background and Aims: The incidence of inflammatory bowel disease (IBD) is increasing worldwide, and many atypical IBDs are being discovered. Recently, a unique type of gastrointestinal ulceration disease with recessive *SLCO2A1* gene mutations was identified and named chronic enteropathy associated with *SLCO2A1* gene (CEAS). We report nine patients with CEAS before adulthood.

Methods: Nine patients diagnosed with CEAS at Seoul National University Children's Hospital were identified. Clinical data of patients were collected. Sanger sequencing was performed on all patients.

Results: Patients were diagnosed at a median age of 16.6 years (SD 6.38 years). The median age at symptoms onset was 6.6 years (SD 5.05 years). Two novel mutations of *SLCO2A1*, c.1329_1344del and c.1350C>G, were identified. Endoscopy revealed multiple small bowel ulcerations in all patients, including terminal ileum lesions in two. Circumferential transmural ulcerations were observed in surgical specimens. Abdominal pain and pale face were the most common symptoms of CEAS. All patients presented anemia, iron deficiency, and nearly normal C-reactive protein levels in the initial laboratory tests. Features of primary hypertrophic osteoarthropathy were observed in three patients. There was no effective medication for CEAS in our patients.

Conclusions: CEAS is a newly discovered, important, chronic IBD. It should be considered as one type of very-early onset IBD. When a patient shows IBD-like manifestations with normal systemic inflammatory markers from young age, screening tests for *SLCO2A1* gene should be considered. Currently, there is no specific treatment for this disease; therefore, further multicenter studies for treatment will be necessary.

Keywords: Inflammatory bowel disease; SLCO2A1 gene, human; Intestine, small

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Contents

Introduction

The incidence of inflammatory bowel disease (IBD) in children is steadily increasing worldwide, and many studies are currently being conducted to determine the genetic susceptibility of IBD.[1-4] Chronic enteropathy associated with the *SLCO2A1* gene (CEAS) is a recently reported rare IBD caused by recessive mutations in *SLCO2A1*.[5, 6] In 1968, Okabe *et al.* first described an unique type of chronic enteropathy with multiple shallow ulcers in the small intestine, called chronic nonspecific multiple ulcers of the small intestine (CNSU).[5, 6] In 2015, Umeno *et al.* found recessive mutations of *SLCO2A1* in some CNSU patients, which is known to encode for a prostaglandin transporter.[7] Subsequently, the disease called CEAS was established.

This disease is characterized by persistent gastrointestinal (GI) bleeding, multiple nonspecific ulcers, and intestinal stenosis.[8, 9] In addition, mutation of *SLCO2A1* is also known as the cause of primary hypertrophic osteoarthropathy (PHO) which was characterized by pachydermia, digital clubbing, and periostosis.[10, 11] Thus it was assumed that there might be patients with both symptoms of the two different diseases observed.

CEAS is considered to be underdiagnosed or misdiagnosed because this disease is not well known, and its characteristics are similar to those of other GI ulceration diseases such as other IBDs, intestinal tuberculosis, and non-steroidal anti-inflammatory drug (NSAID)-associated enteropathy. To date, approximately 50 cases of CEAS have been published.[7, 8, 12-17] All CEAS patients were reported exclusively in Asia and the number is gradually increasing. But there were no reports focused on pediatric cases of CEAS, despite the high possibility of developing the disease at an early age because the disease is caused by gene

mutations. Furthermore, *SLCO2A1* appears to be a new gene locus for very early-onset inflammatory bowel disease (VEO-IBD), but has not been recognized yet globally.[18]

Based on previous studies, we estimated that among patients with atypical chronic enteropathy before adulthood, there would be some patients with CEAS. Therefore, this study was performed to identify patients with CEAS before adulthood in Seoul National University Children's Hospital (SNUCH). Furthermore, through patients diagnosed with CEAS, we evaluated the detailed characteristics of the disease and its clinical course for treatment.

Materials and methods

1. Study participants

In 2018, Umeno *at el.* first proposed clinical criteria for CEAS.[9] Based on this, the diagnosis of CEAS was confirmed by combining symptoms of blood loss from the GI tract, evidence of intestinal lesions by enteroscopy or radiography, and genetic confirmation of *SLCO2A1* mutations.[9] According to these diagnostic criteria, we diagnosed our patients with CEAS.

From January 2018 to April 2021, we performed a molecular genetic test of *SLCO2A1* in patients who were suffering from chronic GI tract-related problems that were ill-treated and inconsistent with typical IBD at SNUCH. From this work, we identified seven patients with *SLCO2A1* mutations and they were newly diagnosed with CEAS. Furthermore, we found two additional patients who had been clinically diagnosed with PHO and had chronic abdominal symptoms. Sequencing of *SLCO2A1* was done to them and recessive mutations were found, so two patients were additionally diagnosed with CEAS. In total, nine patients diagnosed with CEAS at SNUCH, were enrolled. This study was approved by International Review Board (IRB) of Seoul National University Hospital (IRB Number: H-2105-022-1216).

2. Data collection

Clinical data of patients, including sex, body weight, height, age of onset and diagnosis, initial blood lab, response to treatment, and detailed information of *SLCO2A1* variations were collected. Information about GI tract involvement was collected from the endoscopic, radiologic, and histologic findings. All continuous variables in the blood laboratory results were presented as mean with range and

standard deviation (SD), and all discrete variables were presented as numbers with percentages (%). All z-scores of height and weight of patients were analyzed using 2017 Korean national growth charts.[19] Also, we used the dataset of Genome Aggregation Database (gnomAD), version 2.1, to analyze the population frequencies of each *SLCO2A1* mutation revealed.[20] All enrolled patients provided written informed consent for the genetic molecular analysis.

3. Molecular diagnosis

Blood samples were drawn from all enrolled patients for molecular genetic testing of *SLCO2A1*. Sanger sequencing of *SLCO2A1* was performed for all patients. Genomic DNA was isolated from peripheral blood leukocytes from the study subjects. All 14 coding exons of *SLCO2A1* and their intronic flanking regions were PCR-amplified using specific primer pairs. Amplification was conducted over 30 cycles, and the PCR mixtures were separated on 1.5% agarose gels to confirm the size and purity of the PCR products. Subsequently, DNA sequencing reactions were carried out, and the reaction mixtures were analyzed using an ABI3130xl Genetic Analyzer (Applied Biosystems, CA, USA) and Sequencing Analysis v.5.2 software. To predict the functional impact of novel amino acid changes, we carried out molecular analyses of the patients' parents and 100 healthy controls, and compared the results with the patients' results. Additionally, we assessed novel missense alterations using *in silico* prediction algorithms. The suspected variants were further screened using the ExAC browser, gnomAD, or the 1000 Genome Project dataset.

Results

1. Clinical information and endoscopic findings of CEAS

Table 1 shows the clinical information and endoscopic findings of all patients diagnosed with CEAS. Among the nine patients, five were male and four were female. The median age at symptom onset was 6.6 years (range: 1 ¡ 16 years, SD: 5.05), and the median age at diagnosis was 16.6 years (range: 9 ¡ 25 years, SD: 6.38). The median z-score of height at diagnosis was -0.47 (range: -1.76~0.73), and that of weight at diagnosis was ¡ 1.05 (range: -2.35~-0.05). Patients were previously diagnosed with cryptogenic multifocal ulcerous stenosing enteritis (CMUSE), duodenal ulcer, eosinophilic gastroenteritis, and protein losing enteropathy.

All patients were evaluated with esophagogastroduodenoscopy. Most patients except case 4 also underwent colonoscopy. The small bowels of cases 3, 6, 7, and 8 were additionally evaluated with capsule endoscopy (CE). From enteroscopic evaluations, all patients were identified as having ulcerative lesions in the GI tract. Intestinal stricture was found in four patients; some patients were also identified with aphthous lesions and scarring in GI tract. Small intestine lesions were observed in all patients (100%); on the other hand, the colon, esophagus, or gastric body was not affected in any patient (0%). The most affected site of the GI tract was the ileum (66.6%), followed by the duodenum (55.5%), pylorus (33.3%), and jejunum (33.3%). In addition, terminal ileum lesions were found in 2 patients (22.2%).

Intestinal surgery was inevitably performed in five patients due to various reasons such as intestinal stricture, GI tract bleeding, or uncontrolled abdominal pain.

Three patients underwent small bowel resection due to ulcer stricture, and two of them underwent surgery after CE due to capsule retention on the ulcer stricture. Figure 1 and 2 show the characteristics of endoscopic, radiologic, and histologic findings in CEAS patients.

2. Detailed information on the identified *SLCO2A1* mutations

SLCO2A1 is located in chromosome 3, and we identified four different *SLCO2A1* mutations in nine patients. Detailed information on *SLCO2A1* variations was described in Table 1 and 2. Four patients had a homozygous mutation, and five had compound heterozygous mutations. Among the four identified *SLCO2A1* mutations, nucleotide change of c.940+1G>A (intron 7) was the most common mutation in our patients, followed by c.1807C>T (exon13). These two mutations were known as pathogenic variants according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) variant classification guidelines.[21] Two novel mutations, c.1329_1344del and c.1350C>G, were found in our patients. These mutations were both located in exon 10, and they were frameshift and missense mutations, respectively. The c.1329_1344del mutation of S*LCO2A1* was classified as a pathogenic variant, and the c.1350C>G mutation was classified as a likely pathogenic variant, according to the ACMG/AMP variant classification guidelines.[21]

Population frequencies of each *SLCO2A1* mutation were further analyzed by GnomAD database. The allele frequency of the c.1807C>7 variant in East Asian was 5/18,096, which was much higher than that of European (2/111,868). The allele frequency of the c.940+1G>A variant was also much higher in East Asian (6/18,394) than in European (1/113,762). The c.1329_1344del and c.1350C>G

variants have not been identified.

3. Symptoms and laboratory findings of CEAS

Table 3 shows clinical symptoms and initial laboratory findings of all patients with CEAS. Abdominal pain (88.9%) and pale face (75.0%) were the most common symptoms of CEAS. On the other hand, diarrhea (11.1%) and melena (11.1%) were the rarely observed initial symptoms.

Among the nine patients, two had been clinically diagnosed with PHO, and recessive *SLCO2A1* variations were confirmed using Sanger sequencing. They showed typical symptoms of PHO, such as pachydermia, digital clubbing, and periostosis. Figure 3 shows the detailed PHO-related features of case 5. Case 1 also showed pachydermia and digital clubbing, which were found after the diagnosis of CEAS.

In initial laboratory findings, all patients showed low hemoglobin levels (median: 9.50 g/dl, range: 4.4-11.3 g/dl). They also showed relatively low total protein (median: 5.54 g/dl, range: 3.9-7.5 g/dl) and albumin (median: 3.21 g/dl, range: 2.1-4.1 g/dl) levels. The median C-reactive protein (CRP) levels were usually normal or mildly elevated (median: 0.94 mg/dl, range: 0.01-2.87 mg/dl). Their ferritin (median: 13.87 ng/ml, range: <3-75 ng/ml), iron (median: 16.71 ug/dl, range: 9-28 ug/dl), and iron saturation (median: 5.68 %, range: 2.6-13.7 %) levels were extremely low. Occult blood tests were performed in their initial presentation of six patients, and they all showed positive results.

4. Treatment and clinical course

All patients had been treated according to their previous diagnoses. Case 4 have

been diagnosed with duodenal ulcer with active bleeding, so he had been treated with endoscopic hemostasis and *Helicobacter pylori* eradication therapies. Case 5 was also diagnosed with duodenal ulcer, and he had been treated with a H2 antagonist. These two patients currently do not complain of gastrointestinal symptoms and they do not receive CEAS-related treatment.

Other seven patients have been treated with immunomodulatory drugs more than once, and most of the patients took iron supplements for years due to chronic anemia. After correctly diagnosed with CEAS, azathioprine was added to two patients, case 1 and case 2, based on a previous report about efficacy of azathioprine to CEAS.[12]

In our patients, seven patients have been treated with azathioprine. One patient maintains the azathioprine treatment, and another patient discontinued azathioprine due to intestinal perforation at the previous surgical site. The other five patients stopped or changed to other immunomodulatory drugs because they showed no signs of improvement in symptoms and laboratory results. Infliximab and 5-aminosalicylic acid had also been administered to two patients respectively, but they also did not show definite improvement of the disease.

Besides using immunomodulators, two patients had been treated with an elemental diet therapy. It temporarily showed slight improvement in symptoms such as abdominal pain, but long-term elemental diets over 6 months did not show any significant improvement in laboratory findings. In conclusion, none of the treatments, including immunomodulators and elemental diets, showed significant effect on improving symptoms, laboratory abnormalities such as anemia or hypoalbuminemia, and endoscopic findings to CEAS patients.

Table 1. Characteristics and endoscopic features of CEAS

Case	Sex	Age		Z-score at diagnosis		Previous diagnosis (before CEAS)	Involved GI tract[a]	Types of lesion	History and cause of intestinal surgery	*SLCO2A1* nucleotide change	Predicted effect
		Diagnosis	Onset	Height	Weight						
1	Male	19	14	−0.01	−1.29	Eosinophilic gastroenteritis	Pylorus, duodenum, terminal ileum	Ulcer, aphthous lesion	−	c.1807C>T / c.940+1G>A	p.Arg603* / p.Arg288Glyfs*7
2	Female	16	4	−1.47	−1.58	Eosinophilic gastroenteritis	Pylorus, duodenum	Ulcer, stricture	Small bowel segmental resection due to intestinal stricture	c.1807C>T / c.940+1G>A	p.Arg603* / p.Arg288Glyfs*7
3	Female	9	5	−0.68	−0.87	CMUSE	Jejunum, ileum	Ulcer, stricture, aphthous lesion	Small bowel segmental resection due to capsule retention on stricture	c.1807C>T homozygote	p.Arg603*

4	Male	16	16	−0.03	−0.26	Duodenal ulcer	Duodenum	Ulcer	−	c.1807C>T / c.940+1G>A	p.Arg603* / p.Arg288Glyfs*7
5	Male	25	6	0.73	−1.16	Duodenal ulcer	Duodenum, terminal ileum	Ulcer, mucosal nodularity	−	c.940+1G>A / c.1329_1344del	p.Arg288Glyfs*7 / p.Cys444Argfs*31
6	Female	21	4	−0.44	−0.05	CMUSE	Ileum	Ulcer, scar, stricture	Small bowel segmental resection due to capsule retention on stricture	c.940+1G>A / c.1350C>G	p.Arg288Glyfs*7 / p.Cys450Trp
7	Male	10	3	−0.92	−0.42	CMUSE	Jejunum, ileum	Ulcer	Small bowel segmental resection due to persistent intestinal bleeding	c.940+1G>A homozygote	p.Arg288Glyfs*7
8	Male	9	6	−1.76	−1.50	CMUSE	Jejunum, ileum	Ulcer, scar, stricture	−	c.940+1G>A homozygote	p.Arg288Glyfs*7
9	Fem	25	1	0.39	−2.35	Protein	Pylorus,	Ulcer	Subtotal gastrectomy	c.940+1G>A	

	ale					losing enteropathy	duodenum		(Billroth I) due to uncontrolled abdominal pain	homozygote	p.Arg288Glyfs*7

[a]All patients were evaluated with esophagogastroduodenoscopy, and most patients, except case 4, also underwent colonoscopy. The small bowels of cases 3, 6, 7, and 8 were additionally evaluated using capsule endoscopy.

GI, gastrointestinal; CMUSE, cryptogenic multifocal ulcerous stenosing enteritis; CEAS, chronic enteropathy associated with *SLCO2A1* gene.

Table 2. Detailed information of *SLCO2A1* mutations revealed in CEAS patients

Chromosome	Genomic position GRCh37	Site	Nucleotide change[a]	Predicted effect	Molecular consequence	Mutant allele frequency	Classification[b]	gnomAD allele frequency (allele count/allele number)	
								European (non-Finnish)	East Asian
3	133,654,625	Exon 13	c.1807C>T	p.Arg603*	nonsense	5/18	Pathogenic	0.00001788 (2/111,868)	0.0002763 (5/18,096)
3	133,667,736	Intron 7	c.940+1G>A	p.Arg288Glyfs*7	splice site	11/18	Pathogenic	0.00000879 (1/113,762)	0.0003262 (6/18,394)
3	133,664,068	Exon 10	c.1329_1344del[c]	p.Cys444Argfs*31	frameshift	1/18	Pathogenic	0	0
3	133,664,050	Exon 10	c.1350C>G[d]	p.Cys450Trp	missense	1/18	Likely pathogenic	0	0

[a]The Genbank accession number of *SLCO2A1* is NM005630.

[b]Classifications of each mutation were determined according to the ACMG/AMP variant classification guidelines.

[c]Two novel mutations in *SLCO2A1* were identified in CEAS patients in this study.

GRCh37, Genome Reference Consortium Human build 37; gnomAD, Genome Aggregation Database.

Table 3. Clinical presentations and initial laboratory findings of CEAS patients

Presentations	Number (%)
Abdominal pain	8 (88.9)
Pale face	6 (75.0)
Diarrhea	1 (11.1)
Melena[b]	1 (1.11)
Pachydermia[a]	3 (33.3)
Digital clubbing[a]	3 (33.3)
Periostosis[a]	2 (22.2)

Initial lab	Median (range)
Hemoglobin (g/dl)	9.50 (4.4–11.3)
Total protein (g/dl)	5.54 (3.9–7.5)
Albumin (g/dl)	3.21 (2.1–4.1)
CRP (mg/dl)	0.94 (0.01–2.87)
Ferritin (ng/ml)	13.87 (<3–75)
Iron (ug/dl)	16.71 (9–28)
Iron saturation (%)	5.68 (2.6–13.7)

[a]These symptoms are typical characteristics of primary hypertrophic osteoarthropathy.

[b]Melena was observed in only one patient, but occult blood test was performed in six patients initially and all showed positive results.

CRP, C-reactive protein.

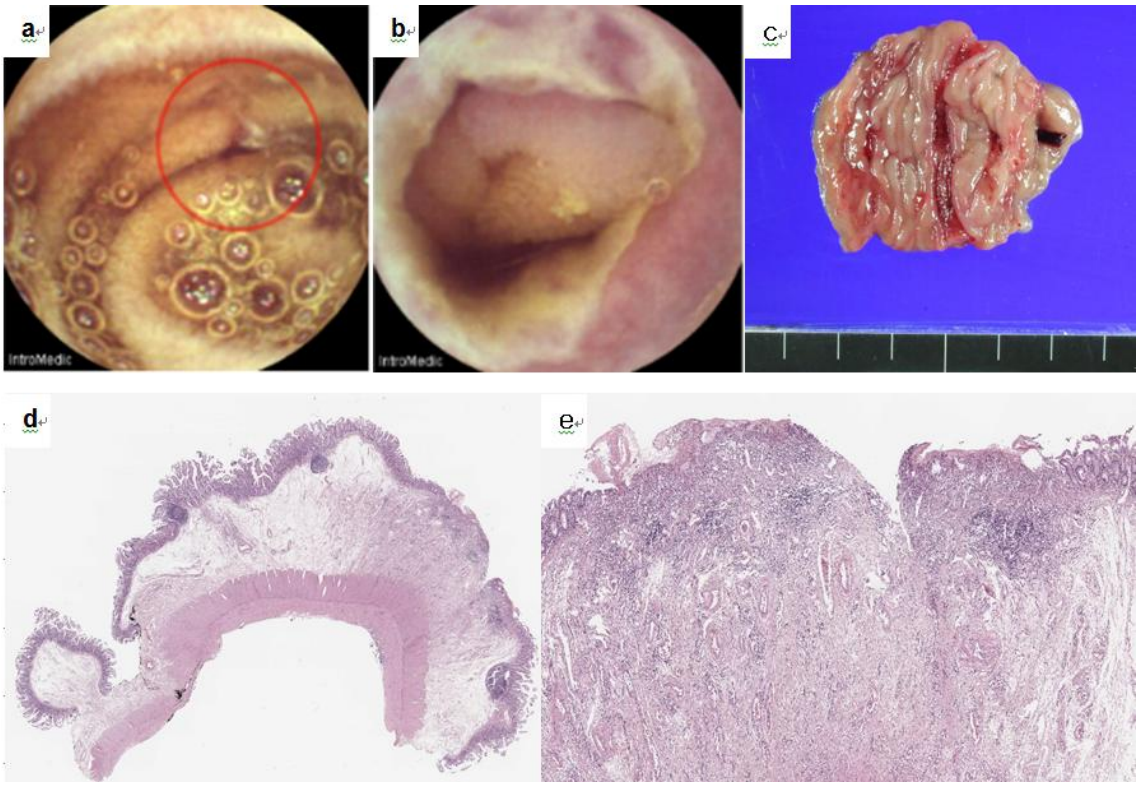

Fig. 1. Capsule endoscopic images [a, b], resected specimen [c], and histologic features [d, e] of CEAS. The figure shows clinical findings of the patient in case 3. She suffered from chronic severe iron deficiency anemia. Capsule endoscopy shows multiple small bowel ulcers [a] and encircling lesions in the small bowel lumen, resulting in luminal narrowing of the proximal ileum [b]. Due to the luminal stricture the capsule was retained in the small bowel; hence, exploratory laparotomy and segmental resection were performed, as shown in [c]. In scan view, focal ulceration with submucosal and mucosal fibrosis can be observed [d]. On magnifying 200 times, focal inflamed granulation tissue can be seen [e]. It does not show characteristic histologic findings of other inflammatory bowel diseases, such as Crohn disease.

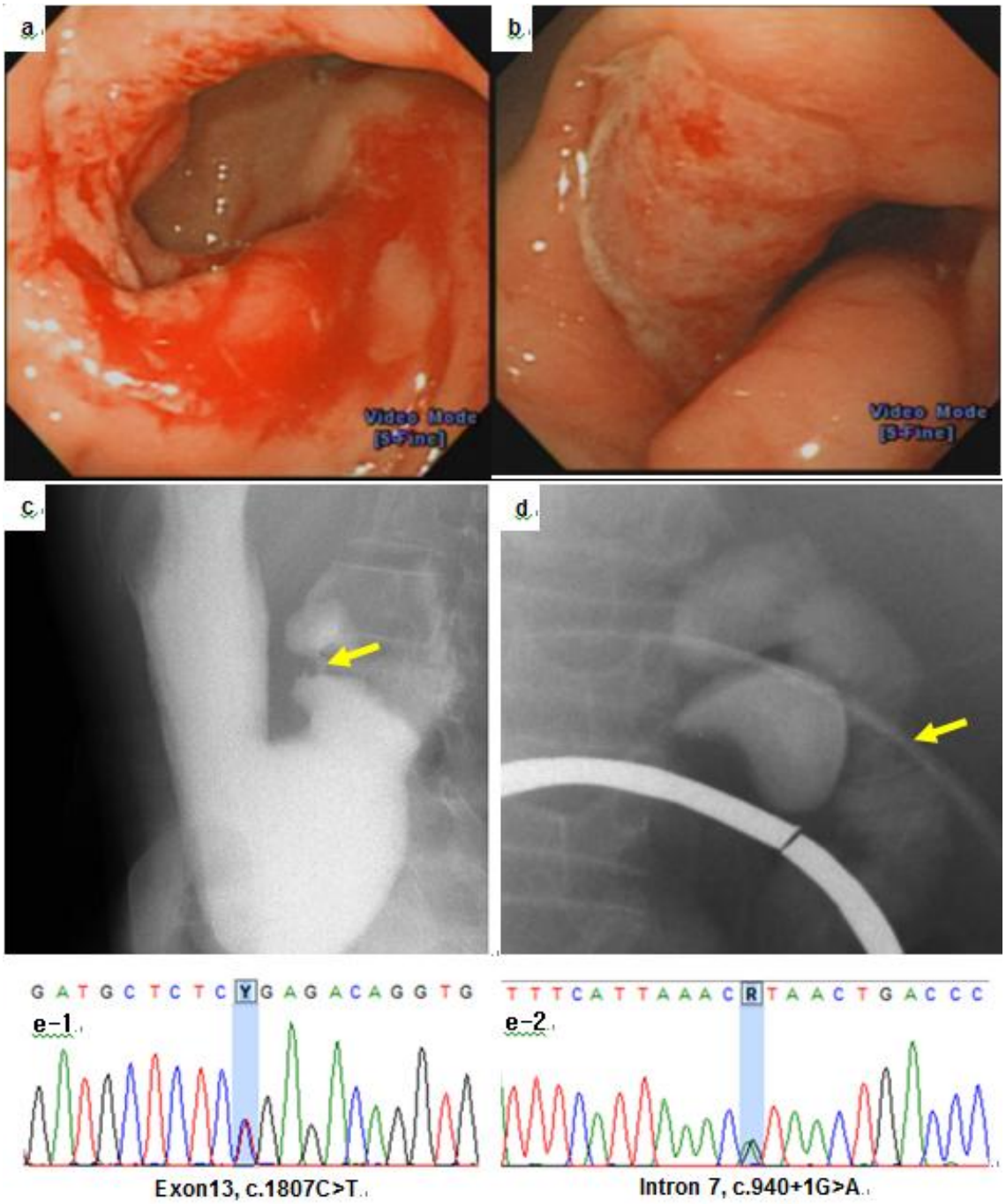

Fig. 2. Esophagogastroduodenoscopy (EGD) images [a, b], upper gastrointestinal study (UGIS) images [c, d], and revealed variations of SLCO2A1 [e-1, e-2]. The figure shows clinical images of the patient in case 2. The patient suffered from chronic abdominal pain and iron deficiency anemia from the age of 4 years. Initial EGD shows broad ulceration with bleeding on the pylorus and duodenal bulb [a, b]. At first, she was treated with a proton pump inhibitor. Two

years later, she still complained of unimproved recurrent abdominal pain and vomiting. UGIS image of that stage shows pyloric stenosis [c] and luminal stricture of the duodenum second portion [d]. Her older brother (case 1) had similar abdominal symptoms and endoscopic findings. At first, they were both diagnosed with eosinophilic gastroenteritis based on histologic finding of eosinophilic infiltration on duodenal ulcer. Recently whole exome sequencing for both siblings was done and same compound heterozygous SLCO2A1 mutations (c.1807C>T and c.940+1G>A) were found, as show in [e-1, e-2].

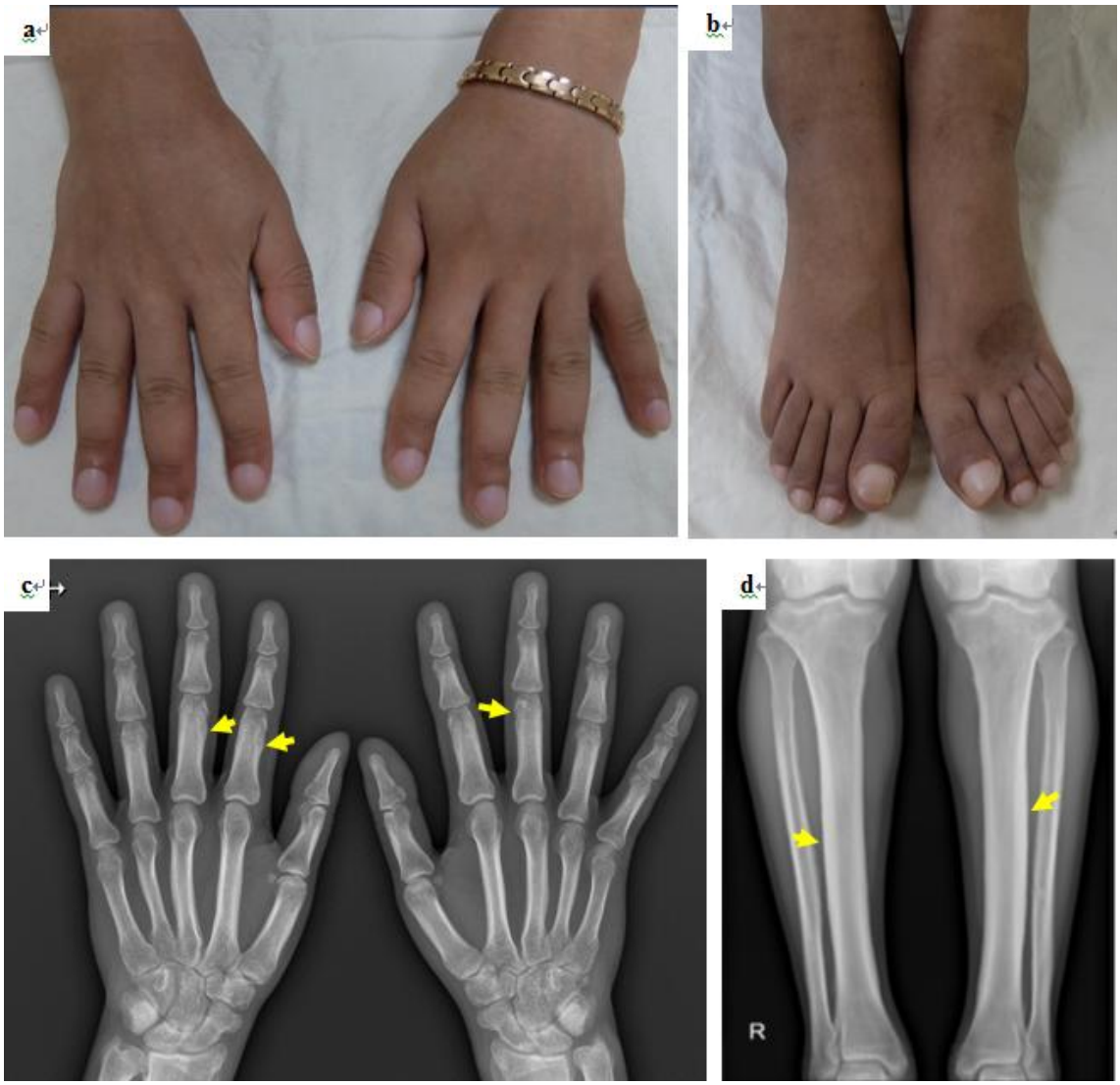

Fig. 3. Clinical features of the patient with primary hypertrophic osteoarthropathy. The patient in case 5 suffered from clubbing of fingers and toes, as shown in [a, b]. About one year before hospital admission, he complained about the coarseness of his face. Hand and lower leg x-ray shows diffuse periosteal thickening along the tubular bones [c, d]. Furthermore, he also had chronic diarrhea and history of duodenal ulcer with active bleeding. Sanger sequencing showed compound heterozygous SLCO2A1 mutations.

Discussion

From this study, we identified nine CEAS patients, and two novel mutations of *SLCO2A1* were found. We also evaluated the characteristics of CEAS and its prognosis of treatment. There are many GI tract diseases that result in small bowel ulcerations and bleeding, including tuberculous enteritis, non-steroidal anti-inflammatory drug (NSAID)-induced enteropathy, intestinal Behcet's disease, and some types of IBD.[22-28] Among them, CEAS is a newly discovered, unique type of inflammatory bowel disease characterized by shallow multiple ulcers and luminal stricture in the small intestine; recessive mutations of *SLCO2A1* are the cause of the disease.[7-9] Although CEAS is gene-causing disease and it can develop from an early age, CEAS has not yet been classified as VEO-IBD and there have been no reports that studied pediatric patients.[9, 18] To date, this is the first report focusing on characteristics of CEAS before adulthood.

All patients in our study showed CEAS-related symptoms with intestinal lesions before adulthood. Many of the patients had chronic GI tract symptoms despite various medications, and this is thought to be the main cause of the relatively short height and low weight for age at diagnosis. The youngest age when symptoms appeared was one year old and the median age at symptom onset in our patients is 6.6 years, which means that CEAS can develop from a very early age, even in infancy. Therefore, *SLCO2A1* should be classified as a new gene locus of VEO-IBD which is defined as inflammatory bowel disease diagnosed or developed 6 years ago.[29]

Enteroscopic and radiologic examinations showed various lesions in the GI tract, from the pylorus to the terminal ileum. Ulcer was the most common lesion, and stricture has also been found quite commonly. A previous article on CEAS from

Japan showed that CEAS could affect almost any small intestine; on the other hand, the terminal ileum was not affected in any patient.[9] However, we found two patients with lesions in the terminal ileum, which had not been identified in the article from Japan. Other case series of CEAS in Chinese have also reported terminal ileum lesions in three patients diagnosed with CEAS.[13] These results indicate that CEAS can damage the entire small intestine, including the terminal ileum. In addition, four patients underwent CE for small bowel evaluation, and two of them had capsule retention on intestinal stricture; for this reason, they underwent small bowel resection. Previously, there were some reports about small bowel resection because of CE retention on stricture in CMUSE, NSAID-induced enteropathy, or other IBD patients.[30-32] From our data, patients with CEAS have a high incidence of intestinal stricture. Therefore, even though CE is a useful tool for evaluating small bowel mucosal lesions, caution should be exercised when evaluating the small intestine with CE in those with suspected CEAS.

Abdominal pain and pale face were the most common symptoms of CEAS. In the blood lab, most of the patients showed anemia, hypoalbuminemia, low iron and ferritin levels, without marked systemic inflammation. Most patients did not show hematochezia or melena, but six patients underwent occult blood tests at initial presentation, and all of them were positive. These results indicate that CEAS mainly shows persistent microscopic hemorrhage from the GI tract rather than symptoms of massive bleeding, and it also results in loss of protein from the intestine and depletion of body iron.

We found two novel pathogenic *SLCO2A1* mutations in CEAS, c.1329_1344del and c.1350C>G, which are frameshift and missense mutations, respectively. The most common pathogenic variant in our study was c.940+1G>A, followed by

c.1807C>T. According to a previous report, these two variants were also most frequently found in CEAS.[8] Furthermore, incidence of both mutations was much higher in East Asian than in European, According to gnomAD dataset.[20] The cause of ethnical differences in the incidence of CEAS is not yet clear, but this is why all CEAS patients were reported in Asian.

Recessive mutations in *SLCO2A1* are also known to cause PHO.[10, 11] Because PHO and CEAS share the same pathogenic gene, it is assumed that patients with one disease may also have the characteristics of the other. There have been some reports about intestinal symptoms of PHO in a family, which might be associated with *SLCO2A1* mutations.[33-35] Recently a case of CEAS with PHO has also been reported in Japan.[36] In our study, cases 4 and 5, who had already been diagnosed with PHO, complained of abdominal pain, and duodenal ulcers were found in both patients through enteroscopy. Case 1 also showed pachydermia and digital clubbing after the diagnosis of CEAS. On the other hand, case 2, who was the younger sister of case 1 and had the same recessive *SLCO2A1* mutations as him, did not show any PHO-related features even though GI tract-related symptoms were much worse than case 1. These features were similar to those of a previous study, which noted that CEAS occurred well in women and PHO occurred well in men.[37] However, the mechanisms are not well known; thus, further studies on gender-specific phenotype differentiation of *SLCO2A1* mutations are needed.

It is unclear how mutations in *SLCO2A1* cause mucosal damage to the small intestine. *SLCO2A1* is known to encode prostaglandin transporter (PGT), which mediates the uptake, release, and clearance of prostaglandin (PG) from cells in multiple organs,[7, 38, 39] and is also expressed in the mucosa of the GI

tract.[40, 41] A previous study showed markedly decreased PGT expression in vascular endothelial cells in the intestine of CEAS compared to that in the control group.[42] Furthermore, PG is known to be associated with both pro-inflammatory and anti-inflammatory effects in the GI tract, and it also contributes to the protection and healing of intestinal mucosa.[41, 43] This indicates that inexpression of PGT in the gastrointestinal mucosa in CEAS induces dysregulation of intracellular PG, which may result in intestinal mucosal damage with a lack of healing ability.

The treatment of CEAS has not been established yet. There was no study about effective treatment of CEAS except one report about efficacy of azathioprine to CEAS.[12] But in our patients, although the number of CEAS patients was small, any medications including azathioprine and infliximab did not show marked effect on symptoms, laboratory, and endoscopic improvement. Because CEAS is not a well-known disease yet, continuous case reporting of CEAS with prospective research on treatment is essential in the future.

This study had several limitations. First, the number of CEAS patients in this report was small because the disease was rare. Second, full endoscopic examinations for evaluating the entire small bowel were not performed in all patients; hence, there is a possibility that other lesions of the small intestine may not be fully identified. In particular, the jejunum and proximal ileum were solely evaluated mainly through CE, and only four patients underwent CE; thus, it is possible that jejunal and ileal lesions of other patients were not found and underestimated. Third, small bowel exploration was performed mainly by esophagogastroduodenoscopy or CE; therefore, histological evaluation through tissue biopsy was limited, and tissues were obtained mainly by surgical

procedures from a few patients. Less invasive methods, such as double-balloon enteroscopy, will be needed for small bowel biopsy in further study.

Conclusion

Our study revealed the clinical characteristics of CEAS in children and adolescents. CEAS is characterized by ulcers, strictures, and microscopic bleeding in the small intestine without systemic inflammation. Because CEAS is a disease caused by a single gene mutation from a very early age, it should be considered as one type of VEO-IBD. CEAS and PHO share the same pathogenic gene; therefore, they can share the symptoms as well. It is important that *SLCO2A1* mutations should be evaluated in patients who show chronic IBD-like features from a young age, with normal systemic inflammatory markers. CEAS is an extremely rare disease and we are still unclear about the pathogenesis and treatment of the disease; therefore, further multicenter study cooperation to explore the pathogenesis and treatment of CEAS is required.

References

1. Doecke, J.D., et al., *Genetic susceptibility in IBD: overlap between ulcerative colitis and Crohn's disease.* Inflammatory bowel diseases, 2013. **19**(2): p. 240-245.
2. de Ridder, L., et al., *Genetic susceptibility has a more important role in pediatric-onset Crohn's disease than in adult-onset Crohn's disease.* Inflammatory bowel diseases, 2007. **13**(9): p. 1083-1092.
3. Jakobsen, C., et al., *Genetic susceptibility and genotype–phenotype association in 588 Danish children with inflammatory bowel disease.* Journal of Crohn's and Colitis, 2014. **8**(7): p. 678-685.
4. Sýkora, J., et al., *Current global trends in the incidence of pediatric-onset inflammatory bowel disease.* World journal of gastroenterology, 2018. **24**(25): p. 2741.
5. Okabe, H. and M. Sakimura, *Nonspecific multiple ulcer of the small intestine.* Stomach and Intestine, 1968. **3**: p. 1539-1549.
6. Matsumoto, T., et al., *Chronic nonspecific multiple ulcers of the small intestine: a proposal of the entity from Japanese gastroenterologists to Western enteroscopists.* Gastrointestinal endoscopy, 2007. **66**(3): p. S99-S107.
7. Umeno, J., et al., *A hereditary enteropathy caused by mutations in the SLCO2A1 gene, encoding a prostaglandin transporter.* PLoS Genet, 2015. **11**(11): p. e1005581.
8. Hosoe, N., et al., *Chronic Enteropathy Associated With SLCO2A1 Gene [CEAS] | Characterisation of an Enteric Disorder to be Considered in the*

Differential Diagnosis of Crohn's Disease. Journal of Crohn's and Colitis, 2017. **11**(10): p. 1277-1281.

9. Umeno, J., et al., *Clinical features of chronic enteropathy associated with SLCO2A1 gene: a new entity clinically distinct from Crohn's disease.* Journal of gastroenterology, 2018. **53**(8): p. 907-915.

10. Niizeki, H., et al., *The complete type of pachydermoperiostosis: a novel nonsense mutation p. E141* of the SLCO2A1 gene.* Journal of dermatological science, 2014. **75**(3): p. 193-195.

11. Zhang, Z., et al., *Exome sequencing identifies SLCO2A1 mutations as a cause of primary hypertrophic osteoarthropathy.* The American Journal of Human Genetics, 2012. **90**(1): p. 125-132.

12. Eda, K., et al., *Successful azathioprine treatment in an adolescent with chronic enteropathy associated with SLCO2A1 gene: A case report.* Medicine, 2018. **97**(41).

13. Huang, H., et al., *Four Variants of SLCO2A1 Identified in Three Chinese Patients with Chronic Enteropathy Associated with the SLCO2A1 Gene.* Digestive Diseases and Sciences, 2020: p. 1-10.

14. Sun, X., et al., *A male Korean who was diagnosed with chronic enteropathy associated with SLCO2A1 (CEAS): case report with literature review.* BMJ open gastroenterology, 2018. **5**(1): p. e000223.

15. Hu, P., et al., *Chronic enteropathy associated with SLCO2A1 gene: A case report and literature review.* Clinics and research in hepatology and gastroenterology, 2019. **43**(5): p. e68-e72.

16. Jimbo, K., et al., *A novel mutation in the SLCO2A1 gene, encoding a prostaglandin transporter, induces chronic enteropathy.* PloS one, 2020.

15(11): p. e0241869.

17. Sun, K., et al., *Multiple small intestinal ulcers with SLCO2A1 and PLA2G4A mutation in a Chinese patient.* Digestive and Liver Disease, 2021. **53**(8): p. 1062-1064.

18. Kelsen, J.R., et al., *North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition position paper on the evaluation and management for patients with very early-onset inflammatory bowel disease.* Journal of pediatric gastroenterology and nutrition, 2020. **70**(3): p. 389-403.

19. Kim, J.H., et al., *The 2017 Korean National Growth Charts for children and adolescents: development, improvement, and prospects.* Korean Journal of Pediatrics, 2018. **61**(5): p. 135.

20. Karczewski, K.J., et al., *The mutational constraint spectrum quantified from variation in 141,456 humans.* Nature, 2020. **581**(7809): p. 434-443.

21. Richards, S., et al., *Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.* Genetics in medicine, 2015. **17**(5): p. 405-423.

22. Somerville, K., G. Faulkner, and M. Langman, *Non-steroidal anti-inflammatory drugs and bleeding peptic ulcer.* The Lancet, 1986. **327**(8479): p. 462-464.

23. Armstrong, C. and A. Blower, *Non-steroidal anti-inflammatory drugs and life threatening complications of peptic ulceration.* Gut, 1987. **28**(5): p. 527-532.

24. Horvath, K.D. and R.L. Whelan, *Intestinal tuberculosis: return of an old*

disease. The American journal of gastroenterology, 1998. **93**(5): p. 692-696.

25. Marshall, J.B., *Tuberculosis of the gastrointestinal tract and peritoneum.* The American journal of gastroenterology, 1993. **88**(7): p. 989-999.

26. Korman, U., et al., *Enteroclysis findings of intestinal Behcet disease: a comparative study with Crohn disease.* Abdominal imaging, 2003. **28**(3): p. 0308-0312.

27. Ruuska, T., et al., *Prospective evaluation of upper gastrointestinal mucosal lesions in children with ulcerative colitis and Crohn's disease.* Journal of pediatric gastroenterology and nutrition, 1994. **19**(2): p. 181-186.

28. Chang, D.K., et al., *Double balloon endoscopy in small intestinal Crohn's disease and other inflammatory diseases such as cryptogenic multifocal ulcerous stenosing enteritis (CMUSE).* Gastrointestinal endoscopy, 2007. **66**(3): p. S96-S98.

29. Uhlig, H.H., et al., *The diagnostic approach to monogenic very early onset inflammatory bowel disease.* Gastroenterology, 2014. **147**(5): p. 990-1007. e3.

30. Tao, E.-W., et al., *Case report of cryptogenic multifocal ulcerous stenosing enteritis (CMUSE): a rare disease may contribute to endoscopy-capsule retention in the small intestine.* BMC gastroenterology, 2019. **19**(1): p. 1-6.

31. Li, F., et al., *Retention of the capsule endoscope: a single-center experience of 1000 capsule endoscopy procedures.* Gastrointestinal endoscopy, 2008. **68**(1): p. 174-180.

32. Pasha, S.F., et al., *Capsule retention in Crohn's disease: a meta-analysis.*

Inflammatory bowel diseases, 2020. **26**(1): p. 33-42.

33. Sethuraman, G., et al., *Familial pachydermoperiostosis in association with protein□losing enteropathy.* Clinical and Experimental Dermatology: Clinical dermatology, 2006. **31**(4): p. 531-534.

34. Kim, W.H., et al., *Crohn's Disease As sociated with Pachydermoperiostosis.* The Korean Journal of Gastroenterology, 1999. **33**(5): p. 723-729.

35. Compton, R.F., et al., *A new syndrome of Crohn's disease and pachydermoperiostosis in a family.* Gastroenterology, 1997. **112**(1): p. 241-249.

36. Tsuzuki, Y., et al., *Chronic enteropathy associated with SLCO2A1 with pachydermoperiostosis.* Internal Medicine, 2020. **59**(24): p. 3147-3154.

37. Castori, M., et al., *Pachydermoperiostosis: an update.* Clinical genetics, 2005. **68**(6): p. 477-486.

38. Kanai, N., et al., *Identification and characterization of a prostaglandin transporter.* Science, 1995. **268**(5212): p. 866-869.

39. Nomura, T., et al., *The two-step model of prostaglandin signal termination: in vitro reconstitution with the prostaglandin transporter and prostaglandin 15 dehydrogenase.* Molecular pharmacology, 2004. **65**(4): p. 973-978.

40. Mandery, K., et al., *Influence of cyclooxygenase inhibitors on the function of the prostaglandin transporter organic anion-transporting polypeptide 2A1 expressed in human gastroduodenal mucosa.* Journal of Pharmacology and Experimental Therapeutics, 2010. **332**(2): p. 345-351.

41. Dey, I., M. Lejeune, and K. Chadee, *Prostaglandin E2 receptor distribution*

and function in the gastrointestinal tract. British journal of pharmacology, 2006. **149**(6): p. 611-623.

42. Yamaguchi, S., et al., *Immunohistochemical differentiation between chronic enteropathy associated with SLCO2A1 gene and other inflammatory bowel diseases.* Intestinal research, 2018. **16**(3): p. 393.

43. Takeuchi, K. and K. Amagase, *Roles of cyclooxygenase, prostaglandin E2 and EP receptors in mucosal protection and ulcer healing in the gastrointestinal tract.* Current pharmaceutical design, 2018. **24**(18): p. 2002-2011.

국문초록

서론: 전 세계적으로 염증성 장 질환을 앓는 환자수가 늘어나고 있고, 많은 비전형적인 증상을 보이는 염증성 장 질환이 발견되고 있다. 최근에 위장관 궤양이 있으면서 *SLCO2A1* 유전자 변이가 확인된 질환이 확인되었고 *SLCO2A1* gene 연관 만성 장병증(chronic enteropathy associated with *SLCO2A1* gene; CEAS) 으로 명명되었다. 이 질환은 전신염증소견 없이 위장관에 여러 궤양 및 협착을 일으키는 것이 특징이다. 이는 유전자 변이로 인해 생기는 질환이기 때문에 최대한 빠르게 발견 및 치료되는 것이 중요하다고 사료되며, 이에 본 연구를 통해 CEAS로 진단된 소아 환자들의 임상양상에 대해 밝히고자 한다.

방법: 서울대학교 어린이병원에서 *SLCO2A1* 변이가 확인된 9명의 환자들을 대상으로 연구하였다. 환자들의 임상 데이터를 수집하였다. 모든 환자들에게 생어 염기서열 분석이 시행되었다.

결과: 환자들은 평균 16.6세(SD: 6.38년)에 CEAS로 진단되었다. 질환이 발병된 평균 나이는 6.6세(SD: 5.05년)였다. 이번 연구를 통해 2개의 새로운 병적 변이(c.1329_1344del, c.1350C>G)가 발견되었다. 다수의 궤양병변이 전 소장을 침범 가능하였고, 복통과 창백한 얼굴이 CEAS의 가장 흔한 증상이었다. 모든 환자들은 빈혈, 철 결핍 소견을 보였고 피검사상 거의 정상적인 C반응단백 수치를 보였다. CEAS 환자들 중 몇몇에게서 일차성 비후성 골관절증(primary hypertrophic osteoarthropathy)의 소견이 확인되었다. 아자티오프린이나 인플릭시맙 등의 어떤 약제도 환자들에게 효과적이지 않았다.

결론: CEAS는 염증성 장 질환 중 최근에 발견된 중요한 만성 염증 질환으로, very early-onset inflammatory bowel disease의 한 종류로 고려되어야 한다. 환자가 염증성 장 질환과 유사한 증상을 보이나 전신염증수치가 정상소견을 보일 경우 *SLCO2A1* 유전자 분석이 꼭 고려되어야 한다. 아직 CEAS의 치료법이 명확하게 밝혀지지 않았으며, 때문에 치료를 위한 다기관 연구가 꼭 필요하다.

주요어: 염증성 장 질환; SLCO2A1 유전자; 소장;

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