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【 CASE REPORT 】

Chronic Enteropathy Associated with *Solute Carrier Organic Anion Transporter Family, Member 2A1 (SLCO2A1)* with Positive Immunohistochemistry for SLCO2A1 Protein

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Abstract:
Chronic enteropathy associated with *solute carrier organic anion transporter family, member 2A1* (*SLCO2A1*) (CEAS) is a rare autosomal recessive hereditary disease characterized by chronic persistent anemia and hypoproteinemia. Its diagnosis typically requires a genetic analysis. The efficacy of immunohistochemical staining with SLCO2A1 polyclonal antibody as a pre-diagnostic tool for CEAS has been previously reported. We herein report a patient with CEAS in whom immunohistochemical staining confirmed SLCO2A1 protein expression. The immunopositive results may have been due to nonsense-mediated RNA decay. As immunohistochemical staining of SLCO2A1 protein may show immunopositive results, a genetic analysis should also be performed when CEAS is strongly clinically suspected.

Key words: video capsule endoscopy, small intestine, balloon-assisted enteroscopy, chronic enteropathy associated with *SLCO2A1* gene

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Introduction

Chronic enteropathy associated with *solute carrier organic anion transporter family, member 2A1* (*SLCO2A1*) (CEAS) is a rare autosomal recessive hereditary disease characterized by chronic persistent anemia and hypoproteinemia due to the loss of protein from intractable small bowel ulcers (1, 2). It was previously referred to as chronic nonspecific multiple ulcers of the small intestine until a mutation of the causative gene, *SLCO2A1*, was identified by Umeno et al. (1). Cases of CEAS are widely documented in Japan, and it is estimated that there are currently 388 afflicted patients (3). The disease concept of CEAS has gradually become widespread in recent years, and cases have also been reported in other Asian countries, such as China (4-7) and Korea (8).

SLCO2A1 encodes a prostaglandin transporter (9), typically SLCO2A1 protein, which is expressed in the vascular endothelial cells of the small intestinal mucosa (1). A genetic analysis is often needed to confirm the diagnosis of CEAS. Previous studies have investigated the efficacy of immunohistochemical staining with SLCO2A1 polyclonal antibody as a pre-diagnostic tool for CEAS (10-12). They reported on the attenuation or disappearance of the immunohistochemical stainability of CEAS for SLCO2A1 protein compared with other inflammatory bowel diseases.

We herein report a patient with CEAS in whom immunohistochemical staining confirmed SLCO2A1 protein expression.

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Case Report

A 45-year-old woman was referred to our hospital because of multiple small undiagnosed intestinal ulcers detected by video capsule endoscopy (VCE). She was taking no regular medications, including non-steroidal anti-inflammatory drugs (NSAIDs). The patient had been born to consanguineous parents but had no relevant medical history. She had no siblings. She had smoked 20 cigarettes/day for 10 years since she was 21 years old but was in the process of quitting. She did not drink alcohol.

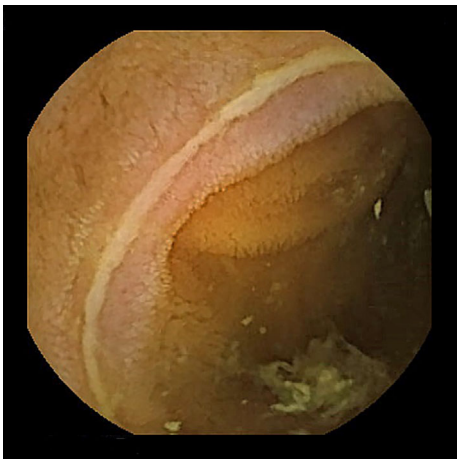

Figure 1. **Image from video capsule endoscopy performed on the patient. Circular and tape-like ulcers were observed in the middle section of the small intestine.**

The patient had been diagnosed with severe iron deficiency anemia (hemoglobin level, 4 g/dL) in her 30s. She underwent esophagogastroduodenoscopy, colonoscopy, and bone marrow aspiration at a nearby hospital, but these procedures did not determine the bleeding source or cause of the anemia. The patient took iron supplements irregularly for six years despite positive fecal occult blood test results. Thus, her previous doctor performed VCE to examine her small intestine. The assessment revealed multiple erosions and ulcers in the middle part of the small intestine (Fig. 1). Subsequently, the doctor performed single-balloon enteroscopy to confirm a pathological diagnosis; however, deep insertion failed to provide diagnostic findings. The patient was therefore referred to our department.

At her first visit with us, no subjective symptoms were observed in the patient, but her palpebral conjunctiva revealed mild anemia. She had no clubbing, thickened skin, or cutis verticis gyrata. The results of her initial laboratory tests are shown in Table. Microcytic anemia and hypoproteinemia were noted (red blood cell count, 4.32×10^6/μL; hemoglobin level, 7.5 g/dL; mean corpuscular volume, 67.1 fL; total

Table. **Laboratory Test Results of the Patient.**

Laboratory parameter	Result (reference range)
White blood cell count	7,800/μL (3,300-8,600/μL)
Erythrocyte count	4.32×10^6/μL (3.86×10^6-4.92×10^6/μL)
Hemoglobin	7.5 g/dL (11.6-14.8 g/dL)
Mean corpuscular volume	67.1 fL (83.6-98.2 fL)
Platelet count	51.7×10^4/μL (15.8×10^4-34.8×10^4/μL)
Prothrombin time	8.9 s
Prothrombin time percentage	>100% (70-140%)
Prothrombin time international normalized ratio	0.83 (0.8-1.2)
Activated partial thromboplastin time	24.9 s (23-36 s)
Total serum protein	4.3 g/dL (6.6-8.1 g/dL)
Serum albumin	2.3 g/dL (4.1-5.1 g/dL)
Serum iron level	11 μg/dL (40-188 μg/dL)
Serum ferritin level	9 ng/mL (8-129 ng/mL)
Serum total iron binding capacity	286 μg/dL (256-407 μg/dL)
C-reactive protein	0.19 mg/dL (0-0.14 mg/dL)
Aspartate aminotransferase	12 U/L (13-30 U/L)
Alanine aminotransferase	8 U/L (7-23 U/L)
Alkaline phosphatase	196 U/L (106-322 U/L)
γ-Glutamyl transpeptidase	7 U/L (9-32 U/L)
Total bilirubin	0.3 mg/dL (0.4-1.5 mg/dL)
Blood urea nitrogen	13.5 mg/dL (8-20 mg/dL)
Serum creatinine	0.46 mg/dL (0.46-0.79 mg/dL)
Serum lactate dehydrogenase	142 U/L (124-222 U/L)
Serum amylase	47 U/L (44-132 U/L)
Serum creatinine kinase	53 U/L (41-153 U/L)
Genetic analysis	
SLCO2A1 gene mutation	c.1807C>T, c.1807C>T

protein concentration, 4.3 g/dL; albumin level, 2.3 g/dL). The patient's inflammatory response was very mild [white blood cell count, 7,800/μL; C-reactive protein (CRP) level, 0.19 mg/dL]. As VCE performed by her previous doctor had revealed erosions and tape-like ulcers in the middle to lower sections of the small intestine (Fig. 1), we performed transoral double-balloon enteroscopy (DBE) on the patient. We achieved deep insertion and found multiple circular and tape-like shallow ulcers, which were identical to the VCE findings, in the middle ileum (250 cm from the ligament of Treitz; Fig. 2). There were no remarkable findings in the stomach or duodenum. A histopathological examination by DBE showed nonspecific mild inflammation and regenerative changes in the mucosa. As CEAS was highly suspected, additional immunohistochemical staining with SLCO2A1 polyclonal antibody (HPA013742; Sigma-Aldrich, St. Louis, USA), vascular endothelial cadherin monoclonal antibody, and 4',6-diamidino-2-phenylindole (DAPI) antibody was performed. The SLCO2A1 protein expression was observed in the vascular endothelial cells of the patient's submucosa (Fig. 3) and in those of a control subject's submucosa (Fig. 4). However, these results did not indicate a probable diagnosis of CEAS. We therefore conducted a further investigation using a genetic analysis to determine the presence or absence of a *SLCO2A1* gene mutation. Interestingly, a *SLCO2A1* homozygous nonsense mutation (c.1807C>T, c.1807C>T) was observed (Fig. 5), which confirmed our diagnosis of CEAS. After the diagnosis of CEAS, iron preparations were given regularly, and since then, the anemia has not progressed. In addition, 18 months after the diagnosis, we performed trans-anal single-balloon enteroscopy, and the small bowel ulcers remained unchanged.

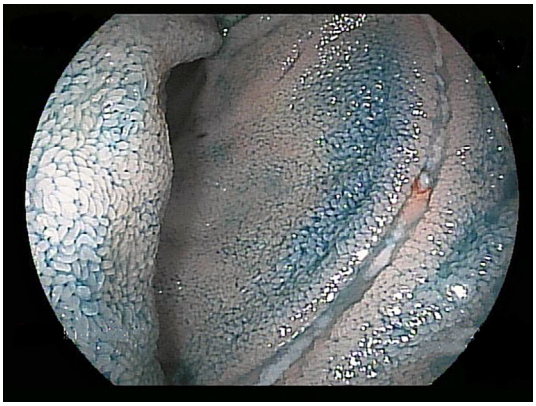

Figure 2. Image from transoral double-balloon endoscopy performed on the patient. Circular and tape-like ulcers, similar to those detected on video capsule endoscopy, were observed in the middle section of the small intestine.

Discussion

Umeno et al. (2) investigated the clinical features of CEAS using a nationwide survey with 46 Japanese patients. CEAS is characterized by an early onset of symptoms (me-

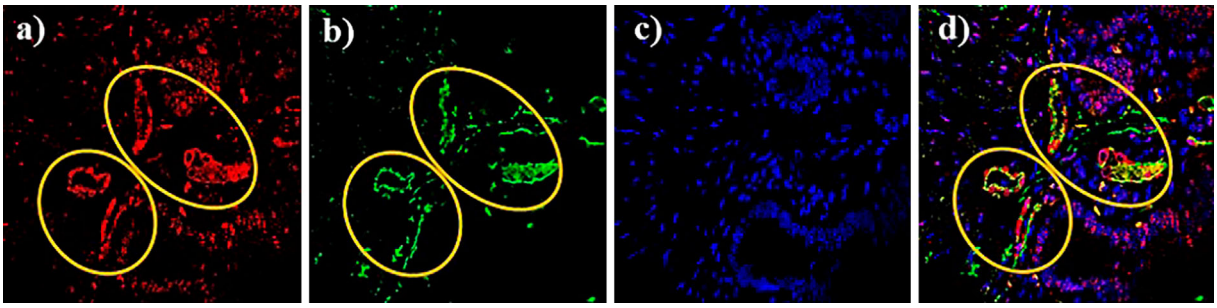

Figure 3. Immunofluorescence staining of the biopsy specimen obtained from the patient's ileum. (a-c) Staining with (a) SLCO2A1, (b) vascular endothelial cadherin, and (c) 4′,6-diamidino-2-phenylindole (DAPI). (d) Metabolic analysis with relative gene expression (MARGE) image.

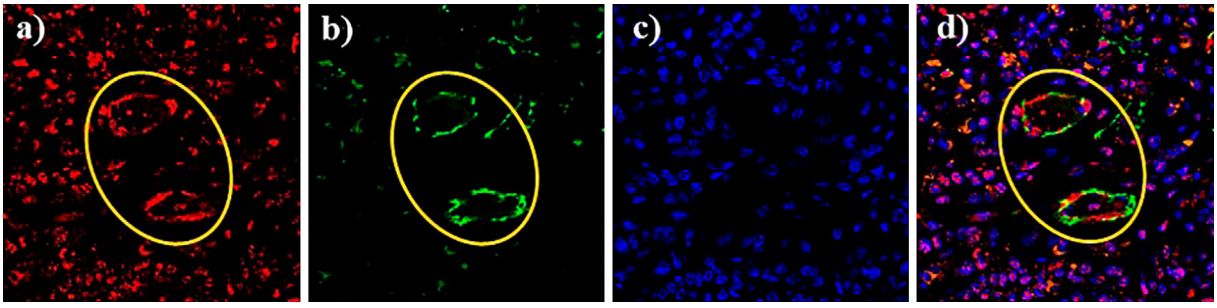

Figure 4. Immunofluorescence staining of a biopsy specimen obtained from a healthy control participant. (a-c) Staining with (a) SLCO2A1, (b) vascular endothelial cadherin, and (c) 4′,6-diamidino-2-phenylindole (DAPI). (d) Metabolic analysis with relative gene expression (MARGE) image.

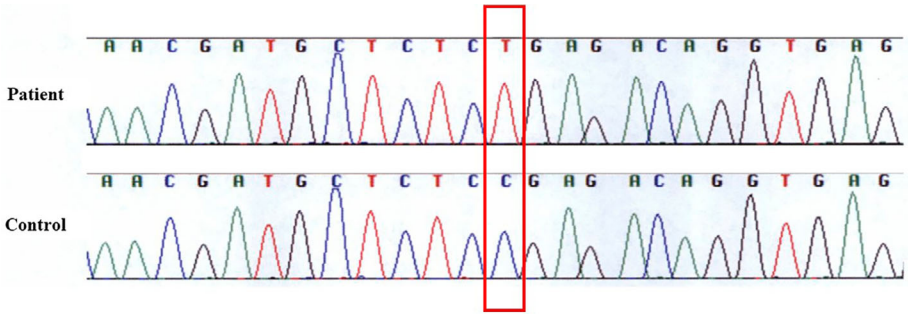

Figure 5. Chromatograms of exon 13 for the *SLCO2A1* mutation in the patient and a healthy control. The homozygous mutation c.1807C>T was detected in *SLCO2A1* in this case.

dian age of onset, 16.5 years old) and female dominance (man-to-woman ratio, 33:13); most patients with CEAS have anemia without hematochezia, revealing a low inflammation marker level (mean CRP, 0.20 mg/dL). The gastrointestinal site most frequently affected by CEAS is the ileum (98%), although terminal ileal ulcers are rare. Lesions are sometimes found in the stomach and duodenum (13-15). Circular or oblique shallow ulcers in the small intestine with clear edges of the ulcers have been detected using enteroscopy (13, 14). Mild digital clubbing or periostosis was previously found in 13 patients (28%), including 5 men who met the major diagnostic criteria of primary hypertrophic osteoarthropathy (2). In our case, the patient was 30 years old at the disease onset; she had anemia without gastrointestinal symptoms, and her CRP level was 0.19. These clinical characteristics are compatible with CEAS.

A histopathological analysis of CEAS has shown that the ulcer remains in the mucosal (Ul-I) or submucosal (Ul-II) layer and does not extend to the muscular layer. In addition, the ulcer site shows nonspecific inflammatory findings, such as mild inflammatory cell infiltration of lymphocytes and plasma cells (2, 15). Curative treatment for CEAS has not yet been established, and symptomatic treatment is the main strategy used in patients. Anemia is temporarily improved by iron replacement therapy and blood transfusions, but it often worsens when treatment ends. In addition, nutritional therapy, such as enteral nutrition, is effective to some extent, and complete parenteral nutrition is also performed when severe malnutrition occurs. In our case, the patient's hemoglobin level was maintained with iron replacement therapy only.

In 2015, Umeno et al. (1) reported that CEAS is caused by a mutation in *SLCO2A1*, which encodes a prostaglandin transporter, based on an exome sequencing analysis. Eleven types of gene mutations have been reported in the literature to date (2), and genetic analyses are crucial for obtaining a definitive diagnosis. SLCO2A1 protein is typically expressed in the vascular endothelial cells of the small intestinal mucosa (10, 12). However, immunohistochemical staining with SLCO2A1 polyclonal antibody has demonstrated attenuation of the SLCO2A1 protein expression in the small intestinal mucosa in CEAS compared with other inflammatory bowel diseases (e.g., Behçet's disease, simple ulcer, and Crohn's disease) (10). The expression rate of SLCO2A1 in biopsy specimen from the gastroduodenum has also been found to be very low relative to Crohn's disease (12).

Immunohistochemical staining of endoscopic biopsy specimens is used as a screening test before a definitive diagnosis of CEAS is made. In our case, antegrade DBE revealed endoscopic findings in the ileum that were characteristic of CEAS; however, immunohistochemical staining demonstrated SLCO2A1 protein expression, indicating an immunopositive result for CEAS. Genetic testing subsequently revealed a mutation in the *SLCO2A1* gene, so we made a definitive diagnosis of CEAS. In contrast, as another screening test, Matsuno et al. reported that prostaglandin E major urinary metabolites (PGE-MUM) were useful for differentiating between CEAS and Crohn's Disease (16). However, in the present case, PGE-MUM measurement was not performed.

The immunopositivity in the presently reported patient may have been due to nonsense-mediated RNA decay. The gene mutation in our case was a homozygous nonsense mutation (c.1807C>T within exon 13, p.Arg603Ter). In general, transcripts expressed from the mutant allele with a truncating mutation undergo nonsense-mediated RNA decay, and the amount of translated protein is minimal. However, due to the location of the mutation within exon 8, which is the penultimate exon, the mutant transcript would escape from nonsense-mediated RNA decay, and the truncated protein would be translated.

Because the mutation is close to the carboxy terminal, the synthesized protein is likely to harbor multiple sites recognized by antibodies. In previous reports (10, 12), two SLCO 2A1-immunopositive cases for immunohistochemical staining were described, both of which had compound heterozygous mutations (c.664G>A, c.1807C>T). Both studies used a polyclonal antibody (HPA013742), as did we. Yamaguchi et al. (10) ascribed the SLCO2A1-immunopositive results of immunostaining using HPA013742 to the full length of the SLCO2A1 protein, which consists of 643 amino acids. In contrast, HPA013742 only recognizes the 83 amino acids of the fifth extracellular domain coded by exons 9-11 (amino acids 431-513). In the current case, the homozygous nonsense mutation (c.1807C>T, c.1807C>T) was found in exon 13. We speculated that the c.1807C>T mutation plays a key

role in SLCO2A1-immunopositive results.

In a previous study on CEAS, the c.1807C>T mutation was found in 21.7% (20/92) of the samples evaluated (2). All of these results indicate that genetic analyses are crucial for making a diagnosis, as some CEAS cases show a SLCO 2A1-immunopositive result on immunohistochemical staining. In our case, negative immunohistological findings were obtained, but CEAS was strongly suspected based on the patient's clinical characteristics, which prompted us to perform additional genetic testing.

In conclusion, immunohistochemical staining of SLCO2A1 protein may show an immunopositive result due to the c.1807C>T mutation. We should suspect CEAS in patients with long-standing iron deficiency anemia and hypoproteinemia, even when they show SLCO 2 A 1-immunopositive results. A genetic analysis should be performed when CEAS is strongly clinically suspected.

The authors state that they have no Conflict of Interest (COI).

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