Distinction between Chronic Enteropathy Associated with the SLC02A1 Gene and Crohn’s Disease

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Background/Aims: We recently identified recessive mutations in the solute carrier organic anion transporter family member 2A1 gene (SLCO2A1) as causative variants of chronic nonspecific multiple ulcers of the small intestine (chronic enteropathy associated with SLCO2A1, CEAS). The aim of this study was to investigate the gastroduodenal expression of the SLCO2A1 protein in patients with CEAS and Crohn’s disease (CD).

Methods: Immunohistochemical staining for SLCO2A1 was performed with a polyclonal antibody, HPA013742, on gastroduodenal tissues obtained by endoscopic biopsy from four patients with CEAS and 29 patients with CD.

Results: The expression of SLCO2A1 was observed in one of four patients (25%) with CEAS and in all 29 patients (100%) with CD (p<0.001). The three patients with CEAS without SLCO2A1 expression had a homozygous splice-site mutation in SLCO2A1, c.1461+1G>C (exon 7) or c.940+1G>A (exon 10). The remaining one CEAS patient with positive expression of SLCO2A1 had compound heterozygous c.664G>A and c.1807C>T mutations.

Conclusions: Immunohistochemical staining for SLCO2A1 in gastroduodenal tissues obtained by endoscopic biopsy is considered useful for the distinction of CEAS from CD.

Key Words: SLCO2A1; Chronic enteropathy associated with SLCO2A1 gene; Crohn disease; Immunohistochemistry

INTRODUCTION

Chronic nonspecific multiple ulcers of the small intestine (CNSU) is a rare autosomal recessive inherited disease characterized by chronic loss of blood and protein through persistent, intractable nonspecific small intestinal ulcers.1-3 The ulcers in CNSU occur predominantly in the ileum, while the terminal ileum is usually intact. The ulcers are multiple (usually >20) and each lesion manifests a shallow and flat ulcer bed surrounded by a discrete margin. The configuration of each ulcer is usually linear or a tall triangle, and the ulcer is aligned circularly or obliquely. The ulcers occasionally fuse, thus showing a geographic configuration.1,5

In 2015, we identified the solute carrier organic anion transporter family member 2A1 gene (SLCO2A1) as the causative gene for CNSU, and we suggested a more appropriate nomenclature of “chronic enteropathy associated with SLCO2A1 (CEAS)” for the disease.6,7 In 2016, Uchida et al.8 conducted a Japanese nationwide clinical and genetic survey of pediatric patients with CEAS, and identified four children with the disease. On the basis of their results, Uchida et al.8 proposed that CEAS should be seriously considered as a differential diagnosis for pediatric patients suspected of having inflammatory bowel disease, especially for those of Crohn’s disease (CD). However, it is often difficult to distinguish CEAS from CD, since both diseases affect the small bowel with intractable chronic ulcers.9,10

Recently, we evaluated SLCO2A1 protein expression in the intestinal tissues of patients with CEAS.11 In the present study, we evaluated the immunohistochemical expression of the SLCO2A1 protein in gastroduodenal tissues obtained by endoscopic biopsy from patients with CEAS and CD to examine whether biopsy specimens can be used for distinguishing these diseases from each other.
MATERIALS AND METHODS

1. Patients

We reviewed the clinical files of patients diagnosed with inflammatory bowel diseases at our institutions between 2001 and 2014. There were 29 patients with CD and four patients with CEAS. Characteristics in three of the four patients with CEAS had been described in the previous study, while all the 29 patients with CD were completely different from those in the previous study. All 33 patients had undergone esophagogastroduodenoscopy with multiple endoscopic biopsies from the normal-appearing gastroduodenal mucosa. In 23 patients with CD and three patients with CEAS, biopsy specimens were obtained from both the gastric and duodenal mucosa. In other four patients with CD and the remaining one patient with CEAS, the specimens were taken only from the stomach. The mean number of the biopsy specimens was 3.6 in CD cases and 3.5 in CEAS cases (Table 1). The diagnosis of CD was based on the established Japanese criteria. All study participants provided written informed consent for this analysis. The study protocol was approved by the ethics committee at Iwate Medical University Hospital (H28-59; August 4, 2016) and Kyushu University (439-06; January 26, 2015). The study was conducted in accordance with the Helsinki Declaration.

2. Genetic analysis of the SLCO2A1 gene

In all four cases of CEAS, mutations in the SLCO2A1 gene were analyzed, as described previously. Briefly, DNA was extracted from peripheral blood using standard methods, and Sanger sequencing of exons 4, 5, 7, 10 and 13 of the SLCO2A1 gene was performed to identify the mutation sites. In the 29 cases of CD, however, mutations in the SLCO2A1 gene were not analyzed.

3. Immunohistochemical analysis of SLCO2A1 protein

In all 33 cases, immunohistochemical staining was performed on 4-µm thick sections cut from each of the formalin-fixed and paraffin-embedded tissue blocks with a polyclonal antibody for SLCO2A1 (HPA013742; Sigma-Aldrich, St. Louis, MO, USA) and a CD31 monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) using the Dako Envision system (Dako, Glostrup, Denmark) according to the manufacturer’s instructions. The immune expression of SLCO2A1 in vascular endothelial cells, as confirmed by CD31 immunostaining, in the respective lesions was determined to be either positive or negative by two independent observers (S. Yanai and N. U.).

4. Statistical analysis

Differences between groups were evaluated with a Fisher exact test, chi-square test or a Mann-Whitney U-test as appropriate. All p-values <0.05 were regarded as statistically significant.

RESULTS

The clinicopathologic features of the study patients are summarized in Table 1. While the median age at diagnosis did not differ between patients with CD (23 years) and patients with CEAS (28 years), the median age at biopsy was significantly younger in CD patients (31 years) than in CEAS patients (63 years, p<0.001). Patients with CD were predominantly male, while CEAS patients were predominantly female. However, the difference was insignificant. Colorectal involvement was confirmed in 27 patients with CD (93%) than in CEAS patients (63 years, p<0.001). Patients with CD were predominantly male, while CEAS patients were predominantly female. However, the difference was insignificant. Colorectal involvement was confirmed in 27 patients with CD (93%), but in none of the patients with CEAS (p<0.001). Laboratory data, including hemoglobin, albumin and C-reactive protein (CRP) levels did not differ significantly between CD and CEAS, though the mean CRP level was higher in CD (2.3 mg/dL) than in CEAS (0.8 mg/dL). Treatment with biologics (infliximab or adalimumab) was carried out in 25 of 29 patients (86%) with CD, while none of the four patients with CEAS (p=0.001) were undergoing treatment with biologics.

SLCO2A1 expression in the gastroduodenal tissues was confirmed in all 29 patients with CD (100%) (Fig. 1). In contrast, SLCO2A1 expression was found only in one of the four patients...
with CEAS (25%, p<0.001) (Table 1, Fig. 2). Both the gastric and duodenal tissues were examined in 23 of 29 patients with CD and in three of four patients with CEAS. Between gastric and duodenal tissues, SLCO2A1 expression pattern did not differ in all the 23 patients with CD and all the three patients with CEAS. In all the 23 patients with CD, SLCO2A1 was positive both in the gastric and duodenal tissues. In all the three patients with CEAS without SLCO2A1 expression, it was negative both in the gastric and duodenal tissues. As shown in Table 2, three of four patients with CEAS without SLCO2A1 expression (cases 1, 2, 4) had a homozygous splice-site mutation in the SLCO2A1 gene, c.1461+1G>C (exon 7) or c.940+1G>A (exon 10). The remaining CEAS patient (case 3) with positive expression of SLCO2A1 had compound heterozygous mutations of c.664G>A and c.1807C>T.

**DISCUSSION**

Development and widespread use of enteroscopy, such as bal-
loin endoscopy and capsule endoscopy, have enabled gastroenterologists to observe mucosal lesions of the whole small bowel. Therefore, the opportunities for diagnosing small bowel diseases have increased. However, the practical and accurate diagnosis of various ulcerative disorders of the small bowel, such as CD, intestinal Behçet’s disease, CEAS, cryptogenic multifocal ulcerous stenosing enteritis and nonsteroidal anti-inflammatory drug induced enteropathy is a matter of debate, because those diseases are often difficult to distinguish from each other by endoscopic findings alone.

CEAS, previously referred to as CNSU, is a rare autosomal recessive inherited disease characterized by chronic blood and protein loss through persistent, intractable and histologically nonspecific small bowel ulcers. The SLCO2A1 gene, which encodes a prostaglandin (PG) transporter, has been identified as a causative gene not only for CEAS, but also for a subtype of CD syndrome. The SLCO2A1 gene, it can be speculated that the families had actually been suffering from CEAS rather than CD. In our previous study, two of 603 patients with a clinical diagnosis of CD had a genetic diagnosis of CEAS. It thus seems possible that cases of CEAS are clinically indistinguishable from each other by endoscopic findings alone.

Compton et al. and Shim and Suh previously reported family pedigrees of patients with CD combined with PHO. On the basis of the above-mentioned identification of the SLCO2A1 gene, it can be speculated that the families had actually been suffering from CEAS rather than CD. In our previous study, two of 603 patients with a clinical diagnosis of CD were found to have compound heterozygous mutations in SLCO2A1, and accordingly, we revised the diagnosis for those two patients as CEAS. It thus seems possible that cases of CEAS are clinically indistinguishable from CD, probably because of apparently indistinguishable clinical manifestations, such as small intestinal ulcers, anemia and hypoproteinemia. Genetic analysis is therefore mandatory for distinguishing CEAS from CD.

It has been confirmed that the SLCO2A1 protein is expressed on the cellular membrane of vascular endothelial cells within the small intestinal mucosa and submucosa in healthy subjects. In our previous study, loss of SLCO2A1 protein expression in the resected intestinal tissues was confirmed in two of three patients with CEAS, while the expression of the protein was positive in all 22 patients with CD or intestinal Behçet’s disease. In the present study, we further confirmed similar findings in the gastroduodenal mucosal tissues obtained by endoscopic biopsy. As a result, loss of SLCO2A1 protein expression was evident in three of four patients with CEAS (75%), but in none of the 29 patients with CD. It thus seems possible that in patients with loss of SLCO2A1 protein expression in biopsy specimens, genetic analysis for the SLCO2A1 gene may not be necessary for the clinical diagnosis of CEAS.

Table 2. SLCO2A1 Mutations and SLCO2A1 Expression in the 4 Patients with CEAS

| Case | Age at biopsy, yr | Sex | Exon | Pattern | Nucleotide change | Mutant allele frequency | Amino acid change | SLCO2A1 protein |
|------|------------------|-----|------|---------|-------------------|------------------------|-------------------|----------------|
| 1    | 57               | Female | 10   | Homozygous | c.1461+1G>C c.1461+1G>C | 2/32 | Splice | Negative |
| 2    | 75               | Female | 7    | Homozygous | c.940+1G>A c.940+1G>A | 19/32 | Splice | Negative |
| 3    | 53               | Female | 5    | Compound | c.664G>A        | 4/32 | Deleterious | Positive |
| 4    | 69               | Male | 7    | Homozygous | c.940+1G>A c.940+1G>A | 19/32 | Splice | Negative |

CEAS, chronic enteropathy associated with SLCO2A1.

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loss through persistent, intractable small bowel ulcers, are essential to screen subjects who are candidates for genetic analysis of SLCO2A1.

In conclusion, immunohistochemical staining for SLCO2A1 in gastroduodenal tissues obtained by endoscopic biopsy may be useful for the distinction of CEAS from CD. With the use of the immunostaining, approximately two-thirds of patients with CEAS could be correctly diagnosed.

CONFLICTS OF INTEREST

T.M. has received grant support from AbbVie, Japan. The other authors declare that they have no conflicting interests.

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