CASE REPORT

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Tufting Enteropathy with *EpCAM* Mutations in Two Siblings

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Tufting enteropathy is a rare autosomal recessive disorder presenting with early-onset severe intractable diarrhea. The epithelial cell adhesion molecule gene (*EpCAM*) has recently been identified as the gene responsible for tufting enteropathy. Based on histology, a diagnosis of tufting enteropathy was made in two Korean siblings. They developed chronic diarrhea and failure to thrive. They had a broad nasal bridge and micrognathia. Duodenal and colonic biopsies showed villous atrophy, disorganization of surface enterocytes, and focal crowding resembling tufts. Protracted diarrhea continued and so cyclic parenteral nutrition was supplied. The sister had juvenile rheumatoid arthritis. Mutation analysis of *EpCAM* identified two compound heterozygous mutations in these siblings: 1) a donor splicing site mutation in intron 5 (c.491+1G>A) and 2) a novel nonsense mutation in exon 3 (c.316A>T, Lys106X). Analysis of *EpCAM* will be useful for genetic counseling and prenatal diagnosis of tufting enteropathy. **(Gut Liver 2010;4:407-410)**

Key Words: Tufting enteropathy; Diarrhea; Epithelial cell adhesion molecule; Mutation

INTRODUCTION

Tufting enteropathy is a rare autosomal recessive disorder presenting with early-onset severe intractable diarrhea. The histological characteristic of tufting enteropathy is the presence of focal epithelial tufts composed of closely packed enterocytes with rounding of the apical plasma membrane, which results in a tear-drop configuration.[1] Some infants have dysmorphic facial features and malformations including choanal atresia, esophageal atresia, or imperforate anus.[2,3] Watery diarrhea develops in the first days after birth and persists despite bowel rest. Growth is impaired, and most patients require total parenteral nutrition indefinitely. In some patients with intestinal failure, intestinal transplantation has been used to treat tufting enteropathy.[4-6]

Since tufting enteropathy was first described in 1994,[7] several cases including a Korean infant have been reported.[6,8-10] The prevalence of tufting enteropathy is estimated at around 1/50,000-100,000 live births in Western Europe.[2] Recently, mutations in the epithelial cell adhesion molecule (*EpCAM*) gene have been identified as responsible for tufting enteropathy.[11] We report two Korean siblings with tufting enteropathy confirmed by genetic analysis.

CASE REPORT

A three-month-old female infant was brought to Seoul National University Children's Hospital having suffered watery diarrhea since she was seven days old together with failure to thrive. The parents are healthy and nonconsanguineous. She was born by normal spontaneous vaginal delivery at 40 weeks of gestation with a birth weight of 3.6 kg. On admission, the child's body weight was 4.5 kg and her height was 54.5 cm (<3rd percentile for age). Stool tests for pathogens and stool electrolyte concentrations were unremarkable. Serum immunoglobulin levels were normal, and circulating autoantibodies were negative. The patient failed to tolerate either casein hydrolysate or amino acid formula. Stool output was up to 150 mL/kg per day. Diarrhea persisted despite bowel rest and total parenteral nutrition. Endoscopic duodenal

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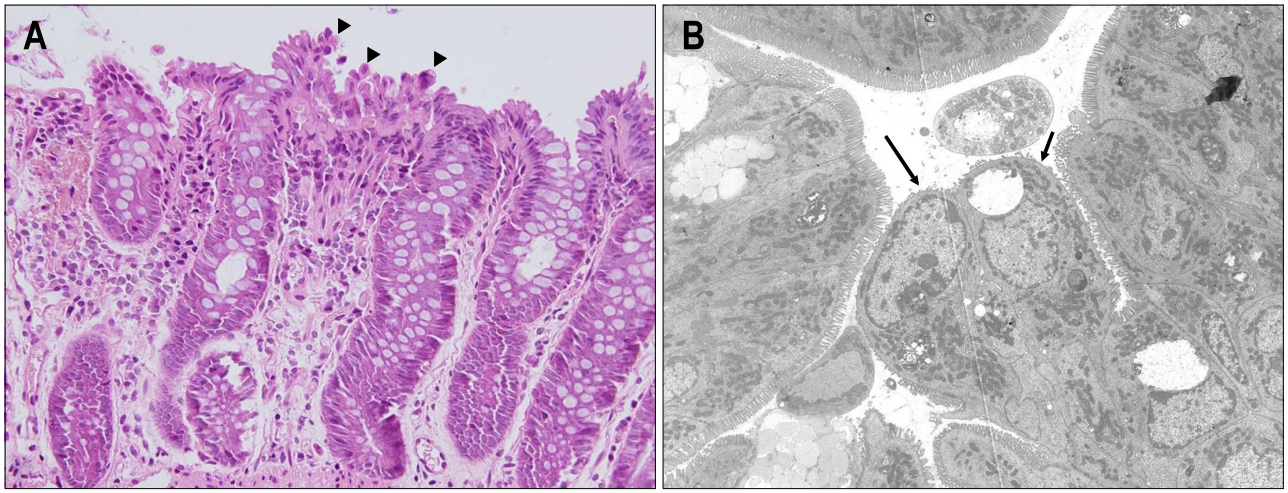

Fig. 1. (A) Mucosal biopsy specimen of the sigmoid colon with tufting (arrowheads) of superficial epithelial cells (original magnification, ×400). Intraepithelial lymphocytes were not elevated. (B) Duodenal mucosal biopsy specimen in the same patient showed decreased number of microvilli of two surface enterocytes (arrows) with structural disorganization, producing a teardrop appearance (original magnification, ×2,000).

and colonic biopsies at three and 12 months of age revealed focal tufting of epithelial cells and disorganization of the surface enterocytes (Fig. 1). Based on this histology, a diagnosis of tufting enteropathy was made. She had a broad nasal bridge and micrognathia. The patient had wooly and easily removable hair. Deficiencies of zinc and selenium were noted, and her hair texture was normalized with zinc and selenium supplementation. At the age of 5 years, pain of both wrists and multiple proximal interpharyngeal joints developed. Swelling and tenderness of involved joints were noted. Radiologic abnormalities were osteoporosis, soft tissue swelling and joint space narrowing. Oligoarticular juvenile rheumatoid arthritis was diagnosed and prednisolone was administered. She has suffered several central-line infections. She is now seven years old, her protracted diarrhea continues, and cyclic parenteral nutrition is supplied. Her height is 107.5 cm and her weight is 15.8 kg (<3rd percentile for age).

The second patient is a brother of the first patient. He was born at 40 weeks of gestational age with a birth weight of 4.2 kg. He presented with vomiting and poor oral intake in the first two weeks of life. At the age of five months, he weighed 3.7 kg. Folate and vitamin B_{12} deficiencies were observed. He had a broad nasal bridge and micrognathia, and developed chronic diarrhea since 6 months of age. A duodenal biopsy at 6 months of age revealed villous atrophy and focal epithelial tufting. Duodenal and colonic biopsies at 32 months of age also showed focal epithelial tufts. He is now four years old, with poor oral intake and persistent diarrhea (stool output up to 30 mL/kg per day), and he is still dependent on parenteral nutrition. His growth and development are normal.

After the informed consent of the patients' parents had been obtained, genomic DNA was extracted from the peripheral blood leukocytes of the children. Mutation analysis of *EpCAM* was performed using PCR amplification and direct sequencing. Compound heterozygous mutations were identified in these siblings. One was a donor splice site mutation in intron 5 (c.491+1G>A) and the other a nonsense mutation in exon 3 (c.316A>T, p.Lys106X). Their mother was heterozygous for c.491+1G>A, and their father was heterozygous for p.Lys106X. Direct sequencing of exon 3 was performed on blood samples from 50 healthy Korean controls, and the novel mutation p.Lys106X was not detected.

DISCUSSION

The abnormal deposition of laminin and heparin sulfate proteoglycan in the basement membrane has been reported in patients with tufting enteropathy.[12] An increase in the number and length of the desmosomes between enterocytes and an abnormal distribution of $\alpha_2\beta_2$ integrin have been observed in pathological studies of tufting enteropathy,[13] which suggests that changes in cell-cell adhesion play a role in the pathogenesis of tufting enteropathy.

EpCAM has been identified as the gene involved in tufting enteropathy. EpCAM is a cell-adhesion molecule originally identified as a marker for carcinoma because it is highly expressed on rapidly proliferating tumors of epi-

thelial origin. EpCAM functions as a typical adhesion molecule and is connected to the actin cytoskeleton.[14] EpCAM interacts directly with claudin 7, a protein required for the formation of tight junctions, implying a role for EpCAM in cellular adhesion.[15] However, recent data have revealed a more versatile role for EpCAM, which is not limited to cell adhesion but includes the migration, proliferation, and differentiation of cells.[16] EpCAM mediates homotypic interactions between intraepithelial lymphocytes and intestinal epithelial cells during the generation of the innate immune system.[17]

The c.491+1G>A mutation found in these two siblings has been reported in Mexican Americans with tufting enteropathy. The homozygous mutation of this splice site is reported to result in the complete deletion of exon 4 and the reduced expression of EpCAM.[11] p.Lys106X is a novel nonsense mutation, which may produce a truncated second epidermal growth factor (EGF)-like repeat in the extracellular domain. The second EGF-like repeat mediates the lateral interactions between EpCAM molecules. The truncation of the EGF-like repeat is known to inhibit the formation of homotypic cell adhesion.[18] The lack of lateral interactions between the molecules may lead to the desmosomal abnormalities observed in tufting enteropathy.

There is some variation in the clinical severity in these siblings, who had the same types of mutations. The sister failed to thrive and her diarrhea was more severe. Factors other than *EpCAM* gene mutations may modify the severity of the disease. The sister was diagnosed as having juvenile rheumatoid arthritis. This is the first report describing juvenile rheumatoid arthritis associated with tufting enteropathy. The siblings had a broad nasal bridge and micrognathia, which was reported in a new syndrome of tufting enteropathy and choanal atresia.[10] The additional features of this syndrome are chronic corneal inflammation, episodic cytopenia, and abnormal hair texture. Wooly and easily removable hair was found in this study, but the abnormal hair was associated with deficiency of zinc and selenium. Further genetic studies are required to investigate the relationship between genotype and phenotype.

The long-term prognosis is variable. Most patients with tufting enteropathy have been treated with long-term parenteral nutrition, which can lead to liver failure, sepsis, and the loss of vascular access. Intestinal transplantation has become a feasible treatment for intestinal failure, with improving results over the past decade. Three patients with tufting enteropathy, including one Korean,[4-6] have undergone small bowel transplantation. A successful pregnancy outcome was reported in a 27-year-old patient with tufting enteropathy.[9]

This is the first report of two siblings with tufting enteropathy attributable to *EpCAM* mutations. The identification of mutations makes it possible to confirm the diagnosis when the results of an intestinal biopsy are not definite. Analysis of the *EpCAM* gene will also be useful for genetic counseling and the prenatal diagnosis of tufting enteropathy.

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