

New Mutations of *EpCAM* Gene for Tufting Enteropathy in Saudi Arabia

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ABSTRACT

Background/Aim: Tufting enteropathy (TE) is a rare cause of congenital intractable diarrhea in children. It often results in an irreversible intestinal failure and total parenteral nutrition (TPN) dependency; eventually, intestinal transplantation may be necessary. Data on TE from the Middle East are scarce; therefore, our aim of conducting this study was to clarify the clinical, histopathologic, and molecular features of TE in Saudi children. **Patients and Methods:** This was a retrospective chart review of four children with TE who presented between January 2011 and December 2013 to King Fahad Specialist Hospital-Dammam (KFSD). The diagnosis of TE was suspected based on characteristic histopathologic intestinal biopsy findings and confirmed by *EpCAM* gene testing. **Results:** Molecular testing identified two novel mutations in the *EpCAM* gene in our patients. These mutations were associated with severe phenotype of the disease characterized by very early onset (median of 2 weeks of life), TPN dependency, and death during early childhood. Two patients died due to central line-related complications. Two patients were referred for intestinal transplantation due to loss of intravenous access in one and progressive liver disease in the other. **Conclusion:** Mutations in *EpCAM* gene in Saudi children are characterized by severe phenotype and poor outcome.

Key Words: Congenital chronic diarrhea, intestinal failure, intestinal transplant, Saudi Arabia, tufting enteropathy

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Tufting enteropathy (TE) was first described in 1994 by Reifen *et al.*^[1] It is one of the rare causes of congenital intractable diarrhea in children characterized by disorganization of the enterocyte surface with focal crowding forming tuft, resulting in irreversible intestinal failure.^[2,3] TE is characterized by a wide range of severity with some patients, with the mild form of disease, can be weaned off total parenteral nutrition (TPN), whereas patients with the most severe form of the disease are dependent on TPN to maintain growth and nutrition and have guarded prognosis without intestinal transplantation.^[4,5] The first genetic characterization of TE was done by Sivagnanam *et al.*, who identified

EpCAM as a gene for TE.^[5] *EpCAM*, which encodes for the epithelial cell-adhesion molecule, is the main gene associated with TE, and maps to chromosome 2p21. However, *SPINT2* gene is another gene that is associated with TE. Salomon *et al.* in their series of 57 TE patients, found *SPINT2* gene in 12 patients.^[4]

The prevalence of TE was estimated at approximately 1/50000–100,000 live births in western Europe.^[2] The prevalence of TE is expected to be significantly higher in areas with a high degree of consanguinity as the population in the Arabian Peninsula; however, data on TE from the countries of the Arabian Peninsula are scarce. Therefore, our aim for conducting this study was to further characterize the clinical, histopathologic, and molecular features of TE in Saudi children.

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PATIENTS AND METHODS

Between January 2011 and December 2013, we retrospectively identified 4 children (3 males) with TE at King Fahad Specialist Hospital-Dammam (KFSH-D) in the Eastern province of Saudi Arabia. The diagnosis of TE was suspected based on characteristic intestinal biopsy findings of villous atrophy and focal epithelial tufting and was confirmed by identification of mutation in the *EpCAM* gene. The electronic medical records were reviewed for patient’s demographics, clinical presentation, diagnostic testing including intestinal biopsy results, as well as genetic analysis, ophthalmologic exam, TPN therapy, complications, and the ultimate outcome. The intestinal biopsies were evaluated by light and electronic microscopy.

RESULTS

Table 1 summarizes the clinical, histopathologic, and molecular characteristics of the 4 patients with TE, as well as the associated morbidity and outcome. All the 4 patients were Saudi, and 3 had consanguineous parents. All the patients manifested a severe phenotype of the disease characterized by very early onset diarrhea (median age of 2 weeks of life), severe failure to thrive, TPN dependency, and death during early childhood. All patients were enrolled in home TPN program for 2–3 years after diagnosis. Intestinal biopsies revealed variable degree of villous atrophy in all the 4 cases [Figure 1] and focal epithelial tufting in 2 [Figure 2]. CD10 immunostain [Figure 3] and electronic microscopy of the intestinal biopsies were performed in 3 patients, which demonstrated an intact microvillous brush border. Extraintestinal manifestations included punctuating keratitis in patient 3.

All the 4 patients had central line-related complications such as multiple infections, that necessitated multiple revisions, and thrombosis and thrombosis of the central veins. Two patients died due to central line-related complications. Two patients were referred for intestinal transplantation due to loss of intravenous access in one (Case 1) and end-stage liver disease due to TPN-associated cholestasis in the other (Case 4).

DISCUSSION

Our report characterizes 4 infants with TE; two of them had novel mutations in the *EpCAM* gene. A striking feature of these mutations is being associated with severe clinical phenotype characterized by TPN dependency and death during early childhood. Patients with TE usually present with onset of intractable diarrhea during early infantile period, severe malnutrition, and TPN dependence. However, TE is

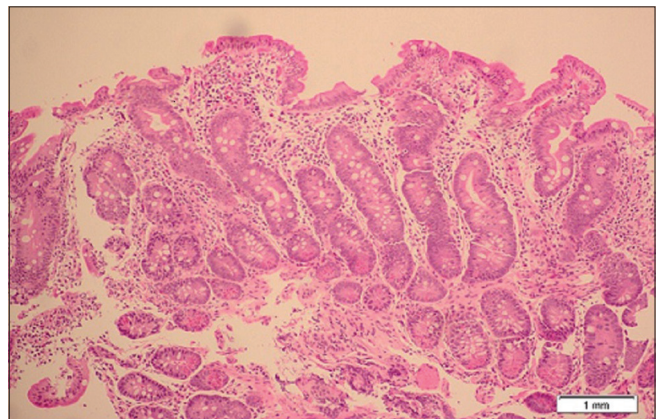


Figure 1: Subtotal villous atrophy and regenerative crypts hyperplasia of duodenal mucosa

Table 1: The clinical, histopathologic, and molecular characteristics of the 4 patients with tufting enteropathy				
Variable	Case 1	Case 2	Case 3	Case 4
Age at presentation	8 months	7 months	2 months	22 months
Gender	Male	Female	Male	Male
Weight at presentation	4.2 kg	3.4 kg	2.6 kg	9.6 kg
Age at onset of diarrhea	Two weeks	Six weeks	Two weeks	First month of life
Family history of TE	Negative	Positive	Negative	undiagnosed neonatal intractable diarrhea
Consanguinity	Yes	Yes	No	Yes
Light microscopy of intestinal biopsy	Mild villous atrophy, focal epithelial tufting, regenerative crypts with new apoptosis bodies, no increased IEL	Subtotal villous atrophy, epithelial tufts, no crypt hyperplasia or increased IEL	Subtotal villous atrophy, no tufts, no crypt hyperplasia or increased IEL	Subtotal villous atrophy, focal epithelial tufting, no crypt hyperplasia or increased IEL
<i>EpCAM</i> gene mutations	A homozygous Mutation c. 499 dup (p.Gln167profs*21)	A homozygous mutation c. 499 dupC (p.Q167fs)	A homozygous deletion of whole gene	A homozygous mutation c. 412C>T (p.Arg138*)
Home TPN Duration	3 years	3 years	2 years	2 years
Response to TPN	Good	Good	Poor	Good
Outcome	Referred for intestinal transplant at age 4 years	Died at age 3.7 years	Died at age 2.2 years	Referred for intestinal transplant at age 2.5 years

IEL: Intraepithelial lymphocytes, TE: Tufting enteropathy, TPN: Total parenteral nutrition

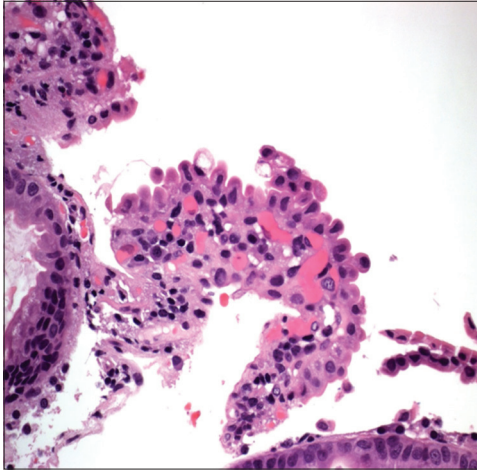


Figure 2: Crowded "tufts" of surface enterocytes

well recognized with its wide spectrum of severity. Varying phenotypes of TE have been described. Complete TPN weaning was possible in some patients, whereas intestinal transplantation was needed in other patients.^[5] Central line and TPN related complications are the main causes of morbidity and mortality in these cases.

The histopathological findings of duodenal and jejunal biopsies on light microscopy (Hematoxylin and eosin stain) are variable degree of villous atrophy and crypt hyperplasia. These histopathologic features are nonspecific and can be seen in other enteropathies due to celiac disease, infections, or cow's milk protein allergy; however, in TE intraepithelial lymphocytes are not increased. The most characteristic feature of TE is the presence of disorganization of surface enterocytes with focal crowding forming tufts. The electron microscopy of intestinal biopsies in TE shows an increase in the length and number of the desmosomes with an increased expression of desmoglein staining of the tight junction. Deposition of laminin is very faint and lamellar in TE, whereas heparan sulfate proteoglycan (HSPG) is overexpressed in the basement membrane. An abnormal distribution of $\alpha 2\beta 2$ integrin has been observed in pathological studies of TE, which suggests that changes in cell-cell adhesion plays a role in the pathogenesis of TE.^[2,3] Staining with the new *EpCAM* antibody clone MOC31 is a reliable test to diagnose TE with a sensitivity and specificity of 100%.^[6] In 2 of our patients, light microscopy did not reveal the focal epithelial tufting that help in the diagnosis of TE. In TE, the histopathologic findings vary from near-normal in early life (showing nonspecific mild villous atrophy) to total villous atrophy and the characteristic epithelial tufts. In addition, the characteristic histopathologic finding (epithelial tufting) can be patchy.^[2,3] Therefore, if TE is suspected, multiple mucosal biopsies should be taken and a repeat endoscopy is warranted.

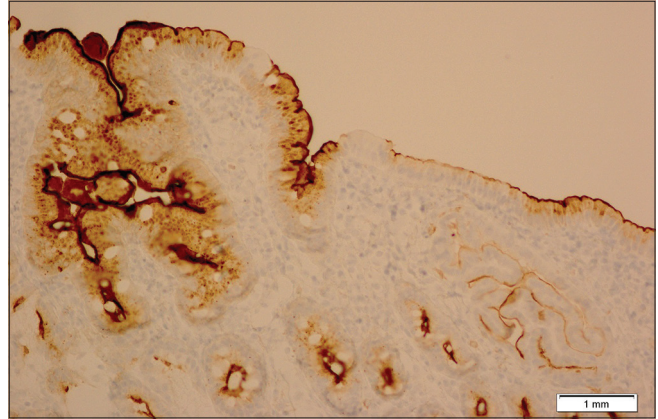


Figure 3: CD10 immunostaining highlights an intact microvillous brush border

Microvillus inclusion disease (MID) is another important congenital enteropathy to consider in an infant with intractable diarrhea. In contrast to TE, MID is characterized by severe atrophy of microvilli at the brush border with apical accumulation of secretory granules in the cytoplasm of enterocytes, and tends to be more severe than TE with persistent life threatening watery diarrhea.^[3] Electron microscopy along with staining of the brush border with periodic acid-Schiff, CD10, polyclonal carcinoembryonic antigen, and alkaline phosphatase can distinguish MID from TE when the histological features on H and E stain slides are inconclusive.^[6,7]

EpCAM belongs to the family of cell adhesion receptors, a large group of molecules mediating cross talk between cells. In addition to its structural function, it is involved in signaling, migration, proliferation, as well as cell differentiation. *EpCAM* is expressed on most epithelial cell membranes, which explains some of the associated extraintestinal manifestations such as keratitis.^[8] Punctate keratitis is very suggestive of TE, but was only observed in one of the 4 patients reported in this series. Early ophthalmologic examination including conjunctival biopsy could help in diagnosis prior to genetic confirmation.^[9]

In our report, all our patients were Saudi in origin with consanguinity in 3 patients. The prevalence of TE seems to be higher in areas with a high degree of consanguinity such as with the population in the Arabian Peninsula,^[3,8] where a founder gene mutation was mapped to exon 5 of the *EpCAM* gene (c. 498insC mutation).^[8] This mutation was predicted to truncate the C-terminal domain necessary to the anchorage of *EpCAM* at the intercellular membrane. Two mutations in our case series, c. 499dup (p.Gln167Profs*21), c. 499dupC (p.Q167fs) have been described previously in Saudi Arabia by Al-Mayouf *et al.*,^[10] and were found to be associated with chronic arthritis and characterized by a less severe disease course and successful weaning off TPN. Our patients (cases 1 and 2) with the same mutations appeared to have a more severe phenotype, which

suggests lack of genotype–phenotype correlation in these particular mutations of *EpCAM* gene. The other two patients (cases 3 and 4) had novel mutations that to the best of our knowledge have not been previously reported.

Parenteral nutrition is the initial therapy for TE, however, timely intestinal transplant is very crucial for those children who develop repeated life threatening sepsis with extensive thrombosis, loss of vascular access, and progressive liver disease.^[3,5,11] The response to TPN is usually satisfactory, however, case 3 in our series continued to show poor response to TPN with no weight gain. Interestingly, this patient had a homozygous deletion encompassing the entire *EpCAM* gene, which might reflect severe genotype of the disease.

In conclusion, we report four cases of TE with two novel *EpCAM* mutations associated with severe phenotype. Further molecular studies of TE are needed to evaluate phenotype–genotype correlation in Saudi Arabia.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Reifen RM, Cutz E, Griffiths AM, Ngan BY, Sherman PM. Tufting enteropathy: A newly recognized clinicopathological entity associated with refractory diarrhea in infants. *J Pediatr Gastroenterol Nutr* 1994;18:379-85.
2. Ko JS, Seo JK, Shim JO, Hwang SH, Park HS, Kang GH. Tufting Enteropathy with *EpCAM* Mutations in Two Siblings. *Gut Liver* 2010;4:407-10.
3. Goulet O, Salomon J, Ruemmele F, de Serres NP, Brousse N. Intestinal epithelial dysplasia (tufting enteropathy). *Orphanet J Rare Dis* 2007;2:20.
4. Salomon J¹, Goulet O, Canioni D, Brousse N, Lemale J, Tounian P, *et al.* Genetic characterization of congenital tufting enteropathy: *Epcam* associated phenotype and involvement of *SPINT2* in the syndromic form. *Hum Genet* 2014;133:299-310.
5. Sivagnanam M, Mueller JL, Lee H, Chen Z, Nelson SF, Turner D, *et al.* Identification of *EpCAM* as the gene for congenital tufting enteropathy. *Gastroenterology* 2008;135:429-37.
6. Ranganathan S, Schmitt LA, Sindhi R. Tufting enteropathy revisited: The utility of MOC31 (*EpCAM*) immunohistochemistry in diagnosis. *Am J Surg Pathol* 2014;38:265-72.
7. Groisman GM, Amar M, Livne E. CD10: A valuable tool for the light microscopic diagnosis of microvillous inclusion disease (familial microvillous atrophy). *Am J Surg Pathol* 2002;26:902-7.
8. Salomon J, Espinosa-Parrilla Y, Goulet O, Al-Qabandi W, Guigue P, Canioni D, *et al.* A founder effect at the *EPCAM* locus in Congenital Tufting Enteropathy in the Arabic Gulf. *Eur J Med Genet* 2011;54:319-22.
9. Roche O, Putterman M, Salomon J, Lacaille F, Brousse N, Goulet O, *et al.* Superficial punctate keratitis and conjunctival erosions associated with congenital tufting enteropathy. *Am J Ophthalmol* 2010;150:116-21.
10. Al-Mayouf SM, Alswaied N, Alkuraya FS, Almehaidib A, Faqih M. Tufting Enteropathy and Chronic Arthritis: A Newly Recognized Association with a Novel *EpCAM* Gene Mutation. *J Pediatr Gastroenterol Nutr* 2009;49:642-4.
11. Paramesh AS, Fishbein T, Tschernia A, Leleiko N, Magid MS, Gondolesi GE, *et al.* Isolated small bowel transplantation for tufting enteropathy. *J Pediatr Gastroenterol Nutr* 2003;36:138-40.