Mutations in Tetratricopeptide Repeat Domain 7A Result in a Severe Form of Very Early Onset Inflammatory Bowel Disease

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Background & Aims: Very early onset inflammatory bowel diseases (VEOIBD), including infant disorders, are a diverse group of diseases found in children younger than 6 years of age. They have been associated with several gene variants. Our aim was to identify the genes that cause VEOIBD. Methods: We performed whole exome sequencing of DNA from 1 infant with severe enterocolitis and her parents. Candidate gene mutations were validated in 40 pediatric patients and functional studies were carried out using intestinal samples and human intestinal cell lines. Results: We identified compound heterozygote mutations in the Tetratricopeptide repeat domain 7 (TTC7A) gene in an infant from non-consanguineous parents with severe exfoliative apoptotic enterocolitis; we also detected TTC7A mutations in 2 unrelated families, each with 2 affected siblings. TTC7A interacts with EFR3 homolog B to regulate phosphatidylinositol 4-kinase at the plasma membrane. Functional studies demonstrated that TTC7A is expressed in human enterocytes. The mutations we identified in TTC7A result in either mislocalization or reduced expression of TTC7A. Phosphatidylinositol 4-kinase was found to co-immunoprecipitate with TTC7A; the identified TTC7A mutations reduced this binding. Knockdown of TTC7A in human intestinal-like cell lines reduced their adhesion, increased apoptosis, and decreased production of phosphatidylinositol 4-phosphate. Conclusions: In a genetic analysis, we identified loss of function mutations in TTC7A in 5 infants with VEOIBD.

*Authors share co-first authorship; †Authors share co-senior authorship.

Abbreviations used in this paper: co-IP, co-immunoprecipitate; EFR3B, EFR3 homolog B; MIA, multiple intestinal atresia; PI4KIIIα, phosphatidylinositol 4-kinase III; SCID, severe combined immunodeficiency; shRNA, short hairpin RNA; TPR, tetratricopeptide repeat; TTC7A, tetratricopeptide repeat domain 7; VEOIBD, very early onset inflammatory bowel diseases; WT, wild type.
Functional studies demonstrated that the mutations cause defects in enterocytes and T cells that lead to severe apoptotic enterocolitis. Defects in the phosphatidylinositol 4-kinase–TTC7A–EFR3 homolog B pathway are involved in the pathogenesis of VEOIBD.

**Keywords:** IBD; Intestinal Atresia; Autoimmunity; Intestine.

Very early onset inflammatory bowel diseases (VEOIBD), including forms of infantile disease, are a diverse group of diseases that are diagnosed before 6 years of age. In contrast to adult-onset IBD, VEOIBD frequently encompasses a unique clinical presentation with severe, colonic disease that often has a poor response to standard therapies, including biologic agents. Recently, several groups, including our own, demonstrated that mutations in *IL10RA/B* genes cause a severe form of VEOIBD, with symptoms consistently developing in infancy. Subsequently, causative variants in *IL10*, *XIAP*, *ADAM17*, and *NCF4*, and association variants in the nicotinamide adenine dinucleotide phosphate oxidase genes *NCF2/RAC2* were identified in VEOIBD patients, suggesting that severe infantile colitis frequently starting immediately after birth might represent a group of heterogeneous monogenic diseases.

Recently, mutations in the tetratricopeptide repeat domain 7 (*TTC7A*) gene were found to cause multiple intestinal atresia (MIA) with severe combined immunodeficiency (SCID), although no details about the intestinal phenotype or function of the *TTC7A* gene were provided. In this report, we describe novel human mutations in the *TTC7A* gene (we termed *TTC7A deficiency*) identified independently by whole exome sequencing that result in severe infantile apoptotic enterocolitis with and without MIA and define the intestinal defects associated with this novel form of VEOIBD.

**Materials and Methods**

**Whole Exome Sequencing**

Genetic studies were carried out with approval from the research ethics board at the Hospital for Sick Children, University of Oxford, Cedars-Sinai Medical Center, and Dr von Hauner Children’s Hospital, LMU Munich. In the index case, whole exome sequencing was performed using the SureSelect Human All Exon 50 Mb kit (Agilent, Santa Clara, CA) with high-throughput sequencing conducted using the Solid 4 System at The Center for Applied Genomics through the Hospital for Sick Children (Toronto, ON) on the complete parent–child trio set. Sanger sequencing was used to verify variant genotypes in the index patient and her family, and 40 infantile patients from the institutions named here were screened for *TTC7A* mutations.

Histologic methods are presented in the Supplementary Material.

**Tandem Mass Spectrometry**

Detailed methods are presented in the Supplementary Materials. Briefly, to identify potential interactors of TTC7A, M2 anti-FLAG-agarose FLAG-agarose FLAG-tagged wild type (WT), E71K, or Q526X TTC7A were transiently overexpressed in HEK293T, immunoprecipitated with FLAG-agarose, and bound proteins were trypsin digested and analyzed by tandem mass spectrometry as described previously.

**Knockdown of Endogenous TTC7A by Short Hairpin RNA**

GIPZ human TTC7A short hairpin RNA (shRNA) (green fluorescent protein tagged) targeting coding regions and green fluorescent protein tagged control shRNA (Thermo Scientific, Logan, UT) were transfected into Henle-407 cells with Lipofectamine 2000 (Life Technologies, Carlsbad, CA). Detailed methods are provided in the Supplementary Materials.

**Apoptosis Analysis**

Confluent cells were starved for indicated time points. Apoptosis was assessed by both measured caspase-3 using Western blotting and cytoplasmic DNA fragments using flow cytometric analysis of Annexin V. Cells were stained with Annexin V-fluorescein isothiocyanate and 7-aminoactinomycin D (BD Biosciences, San Jose, CA) according to manufacturer’s instructions, and samples were run on a BD LSR II analyzer. Apoptotic cells were identified as Annexin V+7-aminoactinomycin D− cells.

**Cell Adhesion Assay**

To evaluate cellular adhesion, approximately 5 × 10^4 cells were seeded on 96-well plates precoated with fibronectin (20 μg/mL; Sigma-Aldrich, St Louis, MO), collagen type I (50 μg/mL; Life Technologies), or bovine serum albumin (5% in phosphate-buffered saline; Sigma) for 60 minutes at 37°C. The wells were subsequently washed with phosphate-buffered saline twice to remove nonadherent cells. After fixation with 4% paraformaldehyde, attached cells were visualized by staining with 1% crystal violet dissolved in 33% acetic acid and were quantified by measuring the absorbance at 570 nm on a Versamax microplate reader (Molecular Devices, Sunnyvale, CA).

** Constructs, Western Blot, Cell Culture, and Immunoprecipitation**

Details of constructs, antibodies, and methods used can be found in the Supplementary Materials.

**Statistical Analysis**

Data are presented as mean ± SD. Experiments were performed with a minimum of 3 replications. Statistical significance between groups was established at *P* < .05 using a 2-tailed Student *t* test. *P* values are indicated in the figure legends and text.

**Results**

**Identification of Apoptotic Enterocolitis in a VEOIBD Patient**

In Family 1 (index case), a female patient born at term to a Caucasian mother and Sudanese father presented with...
high-output secretory diarrhea and hematochezia that started almost immediately after birth, requiring total parenteral nutrition. Colonoscopy demonstrated chronic inflammation with severe friability, exfoliative mucosal changes, and sloughed mucosa within the colonic lumen (Figure 1A). Biopsies taken from the duodenum showed villous atrophy and the duodenum and colon showed glandular dropout with crypt apoptosis and exploding crypts (Figure 1B and C). The severity of the epithelial injury was strikingly reminiscent of acute gastrointestinal graft-vs-host disease and intestine allograft rejection. There was no evidence of perianal disease or dermatological disease. The patient had clinical features of immunodeficiency, including lymphopenia and hypogammaglobulinemia. The patient was treated with 2 mg/kg methylprednisone without significant response. At 11 months of age, she developed respiratory failure and succumbed shortly afterward (see Supplementary Material for details). Autopsy did not show any evidence of bowel atresia, but did confirm widespread severe apoptotic enterocolitis as identified previously by endoscopy.

In Family 2, an infant male was born at 36 weeks’ gestation to non-consanguineous Caucasian parents. Shortly after birth, the infant presented with symptoms of small bowel obstruction due to short-segment jejunal atresia. Despite surgical resection, the intestinal disease progressed.

Figure 1. Histologic and endoscopic characteristics of intestine of patients with TTC7A mutations. (A) Colonoscopy showed severe inflammation characterized by continuous grade 2 colitis and multiple areas of exfoliation and sloughing of the surface epithelium from Family 1. (B) Low-magnification electron micrograph of crypt epithelium from the same biopsy shown in panel C from Family 1. Among regenerating crypt cells, apoptotic cell (white asterisk), enteroendocrine cell (EC), and Paneth cell (PC) are present. Crypt enterocytes showed sparse brush border microvilli (arrow). (C) High magnification of duodenal crypt epithelium showed extensive apoptosis from Family 1 (arrows) (H&E stain). Low (D) and high (E) magnification of cecum epithelium showed extensive apoptosis from Family 2 (arrow) (H&E stain). (F) High magnification of cecum epithelium showed extensive apoptosis from Family 3 (H&E stain).
and the patient was found to have recurrent multiple atretic areas that also required resection. The disease continued to progress and the patient died of cardiac arrest before 3 months of age. A second child from the same family also had jejunal atresia at birth, which was initially resected. The intestinal disease progressed and ultimately resulted in the patient’s death at the age of 19 months. Both children had evidence of immunodeficiency with lymphopenia and T-cell deficiency. Pathologic analysis showed loss of intestinal architecture, focal scarring, and severe inflammation with increased apoptosis reminiscent of graft-vs-host-disease, as described in Family 1 (Figure 1D and E).

Family 3 had 2 infant daughters from consanguineous parents who presented with diarrhea and failure to thrive shortly after birth. Both children had no evidence of overt immunodeficiency and pathologic analysis of colonic biopsies showed similar loss of intestinal architecture, focal scarring, and severe inflammation with increased enterocyte apoptosis (Figure 1F) and areas where surface epithelium was detached, as described in Family 1 and Family 2. The younger girl died before the age of 1 year due to uncontrolled candida sepsis and the older girl is presently being partially treated with total parenteral nutrition (see Supplementary Material for details; summarized in Table 1).

**Whole Exome Sequencing**

Whole exome sequencing of Family 1 resulted in >80 times coverage of exomes and the subsequent identification of a nonsynonymous variant in exon 2 inherited from the father, and a nonsense mutation in exon 14 inherited from the mother in the TTC7A gene (Figure 2A). The nonsynonymous mutation in exon 2 at c.211 G>A resulted in a glutamic acid to lysine substitution at amino acid position 71 (p.E71K; rs147914967). The mutant allele is not found in either the National Center for Biotechnology Information database or the 1000 Genomes database, and is only found in 1 heterozygous allele from 6503 healthy individuals genotyped in the National Heart, Lung, and Blood Institute’s Exome Sequencing Project (http://evs.gs.washington.edu). The mutation was predicted to be highly deleterious with a Polyphen1 score of 0.99 and is located in a highly conserved α-helical region (Figure 2D and Supplementary Figure 1). The second TTC7A mutation in exon 14 at c.1944 C>T transition resulted in a nonsense mutation causing the premature termination of the protein at amino acid 526 (p.Q526X). This nonsense mutation has not been described previously in the datasets mentioned.

In siblings from Family 2 with severe apoptotic enterocolitis, we also identified heterozygous TTC7A mutations. The c.844-1 G>T mutation in the splice acceptor site of exon 7 was inherited from the mother and a c.1204-2 A>G mutation in splice acceptor site of exon 10 was inherited from the father (Figure 2B). These TTC7A mutations were predicted to result in loss of the splice acceptor sites for both exons 7 and 10, respectively, and causing premature stop codons that would disrupt tetratricopeptide repeat (TPR) domains (Figure 2D). These splice mutations have not been previously described.

<table>
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<th>Patient</th>
<th>Age at presentation</th>
<th>Sex</th>
<th>CS</th>
<th>Clinical features</th>
<th>TTC7A mutation</th>
<th>TTC7A mutated protein</th>
<th>Outcome</th>
<th>Immune workup</th>
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<td>p.Q526X</td>
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<td>Normal</td>
<td>Alve, TPN for partial control</td>
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AE, apoptotic enterocolitis; CS, consanguinity; TPN, total parental nutrition.

**Table 1. Summary of TTC7A Mutations and Clinical Features**
in the datasets mentioned and it is likely that these mutations will result in nonsense-mediated decay of the TTC7A messenger RNA.

In siblings from Family 3 with severe apoptotic enterocolitis, we identified a homozygous nonsynonymous mutation in exon 20 at c.2494 G>A (Figure 2C) that resulted in an alanine to threonine substitution at amino acid position 832 (p.A832T). The mutation was predicted to be highly deleterious with a Polyphen14 score of 0.99 and is located in a highly conserved region of the ninth TPR domain (Figure 2D; Supplementary Figure 1) and has not been described previously in the datasets mentioned (see Table 1 for a summary).

Functional Analysis of TTC7A Mutations in Enterocytes

Immunostaining of TTC7A from healthy human control intestinal tissue (duodenum, ileum, and colon) showed that TTC7A was strongly expressed in enterocytes with areas of discreet localization at the plasma membrane and only few lamina propria cells stained positive (Figure 3A). This observed pattern of intestinal expression suggests a primary role for TTC7A in enterocyte homeostasis. To determine if the mutation identified in Family 1 and Family 3 resulted in abnormal TTC7A cellular localization, we transiently transfected Caco-2 cells with Myc-tagged WT, E71K, Q526X, and A832T TTC7A. Immunostaining using anti-Myc antibody demonstrated that E71K, Q526X, and A832T TTC7A mutants appeared to accumulate in cytoplasmic puncta, and the WT-TTC7A localized diffusely in the cytoplasm (Figure 3B). In addition, biopsies from the Family 2 patient with the TTC7A splice acceptor site mutations predicted to result in complete loss of the protein showed loss of TTC7A in enterocytes, as expected, indicating that these mutations might result in nonsense-mediated decay of the TTC7A messenger RNA (Figure 3C).

Knockdown of TTC7A by shRNA resulted in loss of cobblestone morphology, typical of human Henle-407 cells with the development of fibroblastoid morphology with spindle-like features (Figure 3D and Supplementary Figure 2). In addition, overexpression of E71K, A832T, and Q526X TTC7A in Caco-2 cells demonstrated cytoplasmic accumulations of Myc-TTC7A in addition to disrupted cortical actin staining suggestive of adhesion defects or loss of cellular polarity (Supplementary Figure 3). Reduced expression of TTC7A in the enterocytes also resulted in detachment during trypsinization (Figure 4A), impaired adhesion to collagen and fibronectin (Figure 4B), and increased apoptosis, as measured by caspase-3 (Figure 4C) and Annexin V (Figure 4D). These cellular changes are reminiscent of the apoptosis and mucosal exfoliation described in our patient.

TTC7A Binding Partners

Tandem mass spectrometry was performed on proteins co-immunoprecipitated (co-IP) from HEK293T cells expressing human TTC7A, with the aim of identifying TTC7A binding partners. Isolated proteins were digested with trypsin to generate peptide fragments and analyzed by tandem mass spectrometry (Supplementary Figure 4). To
refine this list to TTC7A binding partners, spectral hit counts were compared between WT TTC7A and the E71K mutation samples, and determined that phosphatidylinositol 4-kinase IIIα (PI4KIIIα) protein fragments were able to co-IP with WT TTC7A, but were significantly reduced with E71K TTC7A mutation (Figure 5A and Supplementary Figure 4). This PI4KIIIα and TTC7A interaction was supported by a weighted coexpression network\textsuperscript{15} from small bowel gene expression data demonstrating that Ttc7a falls within a subnetwork (module) of the mouse small bowel network that included P4kca (murine form of PI4KIIIα) (Supplementary Tables 1–4; Supplementary Figure 5). The additional hits identified in the tandem mass spectrometry screen (Supplementary Figure 4) implicated several proteins associated with ubiquitination pathways, including E3 ligases (HUWE1, HECTD1, UBR5), and proteins that function in the ubiquitin-proteasome system (USP9X, PSMD1, VCP).

**Loss of TTC7A Results in PI4KIIIα Dysfunction**

As TTC7A has been implicated previously in PI4KIIIα regulation in yeast,\textsuperscript{16,17} and confirmed through the tandem mass spectrometry and network analysis of mouse small bowel, we next confirmed through co-IP experiments that TTC7A and PI4KIIIα interacted in human cell lines. We found that Myc-Flag-tagged WT TTC7A was able to co-IP PI4KIIIα, indicating that these proteins interact either directly or indirectly in a larger complex (Figure 5B). We also observed reduced co-IP of PI4KIIIα with the TTC7A Q526X and E71K mutated proteins identified in Family 1 and the A832T TTC7C mutation identified in Family 3 (Figure 5B). As the splice variants identified in Family 2 were assumed to be unstable, we would predict that the gene product of these TTC7A mutations would also not bind to PI4KIIIα.

We next examined human PI4KIIIα in intestinal tissue of healthy controls and found that PI4KIIIα was abundantly expressed in both enterocytes and immune cells, including lymphocytes (Figure 5C, left panel). In our patients with TTC7A deficiency, the severe disruption of the bowel architecture with sloughing of the majority of enterocytes made interpretation of PI4KIIIα localization difficult; however, in areas with relatively preserved epithelial architecture, we observed overall reduced PI4KIIIα expression in a patient from Family 2 (while lamina propria expression was preserved; Figure 5C). To confirm these results, we transiently co-transfected TTC7A and TTC7A shRNA into Henle-407 cells and observed a reduction in PI4KIIIα (Figure 5D and E). These results indicate that loss of TTC7A resulted in aberrant subcellular localization of PI4KIIIα in enterocytes.

Finally, we determined that knockdown of TTC7A in human Henle-407 cell lines resulted in decreased phosphatidylinositol 4-phosphate, the end product of PI4KIIIα enzyme, in both the cytoplasm (Figure 5E) and at the plasma membrane (Figure 5F). Together these results indicate that TTC7A is required for PI4KIIIα localization to the plasma membrane and that TTC7A deficiency results in loss of PI4KIIIα signaling in enterocytes.

**Discussion**

Our index case (Family 1) had severe infantile apoptotic enterocolitis with a presentation significantly different from previously described cases of VEOIBD with IL10 and IL10R mutations that are invariably present with colitis and peri-anal disease.\textsuperscript{4,5,16–20} The severe enterocolitis with friability and exfoliative mucosal changes along with villous atrophy, gland dropout, and crypt apoptosis led to our genetic exploration through whole exome sequencing and identification of TTC7A as the causative gene.

The TPR domain is defined by a degenerate consensus sequence of 34 amino acids\textsuperscript{21} and 4 of the 5 TTC7A mutations found in our patients resulted in disruption of these TPR domains. TPR domains mediate protein–protein interactions and the assembly of multi-protein complexes that are involved in the regulation of cell cycle, transcription, and protein transport.\textsuperscript{22} Our tandem mass spectrometry and intestinal network experiments demonstrated an association between TTC7A and PI4KIIIα that was previously only described in yeast.\textsuperscript{16,17} In yeast, the TTC7 ortholog, YPP1, is essential and has been shown to rescue a lethal α-synuclein (αSyn-A53T) yeast mutant.\textsuperscript{23} Ypp1 (TTC7) directly binds to Stt4 (PI4KIIIα), and this binding is critical to maintaining phosphatidylinositol 4-phosphate levels and PI4KIIIα stability at the plasma membrane.\textsuperscript{16,17} In addition, in yeast, the phenotypes of YPP1 (TTC7) and STT4 (PI4KIIIα) conditional mutants are identical and both mutants result in cell wall destabilization and defective organization of actin. Overexpression of STT4 (PI4KIIIα) also suppresses the temperature-sensitive growth defect observed in YPP1 (TTC7) mutants.\textsuperscript{17} The role of TTC7A in PI4KIIIα recruitment to the plasma membrane was also recently confirmed in mammalian cell lines\textsuperscript{24} and we demonstrate for the first time that TTC7A and PI4KIIIα directly interact in human cell lines. Because TTC7A is required for proper localization of PI4KIIIα at the plasma membrane,\textsuperscript{24} we propose that TTC7A mutations result in disease through loss of PI4KIIIα at the plasma membrane and subsequent reduction of phosphatidylinositol 4-phosphate that is required for cell polarity and survival. In support of this model, down-regulation of PI4KIIIα results in increased apoptosis\textsuperscript{25} and, in addition, intestinal-specific murine knockout of P4kca (PI4KIIIα) results in a strikingly severe intestinal phenotype with widespread mucosal epithelial degeneration\textsuperscript{26} reminiscent of our patients with TTC7A mutations. Therefore, our results demonstrate a direct interaction between PI4KIIIα and TTC7A. And similar to the phenotype observed in TTC7A-deficient patients, TTC7A knockout in human intestinal-like cell lines resulted in decreased adhesion and increased apoptosis. These results indicate that disruption of the PI4KIIIα–TTC7A pathway results in a combined T-cell and enterocyte defect that results in the intestinal phenotype described here (Figure 6).

The EFR3 homolog B (EFR3B; ENSG0000084710) gene product EFR3B tethers TTC7A (and TTC7B) to the plasma membrane and is essential for both TTC7A and PI4KIIIα function.\textsuperscript{24} In addition, knockdown of EFR3B results in the loss of both TTC7A and PI4KIIIα at the plasma membrane.
and is critical for PI4KIIIα signaling.24 Interestingly, several EFR3B single nucleotide polymorphisms located both in the EFR3B gene and its flanking regions were reported to be associated with Crohn’s disease (http://www.ibdgenetics.org; Supplementary Table 5; lead single nucleotide polymorphism rs1077492; $P = 1.9 \times 10^{-14}$, odds ratio, 1.11). This locus on chromosome 2 at 25.12 Mb was recently reported in the International Inflammatory Bowel Disease Genetics Consortium meta-analysis as an IBD locus.27 In silico analyses carried out by the International Inflammatory Bowel Disease Genetics Consortium suggested ADCY3 as a potential candidate at this locus27; however, EFRB3’s role in regulating PI4KIIIα-TTC7A implicates EFRB3 as a plausible causative IBD gene at this locus and that this...
Figure 4. (A) Impaired cell adhesion in TTC7A-depleted cells. The total dissociation time, defined as the time required for complete dissociation of all cells from the tissue culture plate, was markedly reduced in TTC7A-depleted Henle-407 cells compared with control cells. Dissociation assay (n = 6, biological replicates; Student t test, *P = .0022). (B) Impaired cell adhesion to collagen and fibronectin in TTC7A-depleted cells. Cell adhesion assays were performed using crystal violet staining. Control and TTC7A-depleted cells were seeded on 96-well plates coated with either collagen or fibronectin. Adhesion was assessed on the basis of optical density (OD) at 570 nm. n = 3; adhesion assay (n = 3) biological replicates, Student t test, **collagen: P = .027 and *fibronectin: P = .0077. (C) TTC7A-depletion in Henle-407 cells results in greater caspase-dependent apoptosis. To investigate the impact of TTC7A suppression on the induction of apoptosis, the activation of caspase-3 was measured by Western blot. Specific cleavage of pro-caspase-3 (32 kDa) into the active caspase-3 fragments (17 kDa) was increased in cells serum starved for 24 and 48 hours. n = 3; P = .012, analysis of variance. (D) TTC7A-depletion in Henle-407 cells results in greater apoptosis measured by flow cytometric analysis of Annexin V. To examine the significance of TTC7A suppression, after loss of attachment, flow cytometric analysis was conducted to quantify the extent of apoptosis in cells starved for 24 and 48 hours. Cells were stained with Annexin V-phycocerythrin and 7-aminoactinomycin D (viability marker); apoptotic cells were identified as Annexin V+ 7-aminoactinomycin D– cells. In TTC7A-depleted cells, the proportion of cells in early apoptosis increased to approximately 4.5% at 24 hours and 11.2% at 48 hours of serum-starvation compared to 1.3% (24 hours) and 4.1% (48 hours) in control cells. Annexin V apoptosis assay n = 3 biological replicates, Student t test, fetal bovine serum (FBS), 24 hours: *P = .0018; FBS, 48 hours: *P = .0076; serum starved, 24 hours: *P = .021; serum starved, 48 hours: *P = .0034. BSA, bovine serum albumin.

Figure 3. Functional TTC7A enterocyte studies. (A) TTC7A expression in intestinal enterocytes. Immunofluorescence microscopy performed on human tissue sections immunostained using anti-TTC7A antibody (and 4′,6-diamidino-2-phenylindole [DAPI]) demonstrates TTC7A expression in enterocytes within the duodenum, ileum, and colon. Lower inset panels represent zoomed images of the corresponding panel above. Scale bars = 100 μm. (B) E71K, Q526X, and A832T mutations in TTC7A. Caco-2 cells were transiently transfected with Myc-tagged WT, E71K, Q526X, and A832T TTC7A, immunostained using anti-Myc antibody and visualized using confocal microscopy. Negative control panels represent staining with secondary antibody only. The control plasmid represents an empty vector sham transfection. Scale bars = 25 μm. (C) TTC7A in enterocytes from patient cecum (Family 2). Immunofluorescence microscopy was performed on TTC7A-immunostained (and DAPI) cecal tissue sections from both control and patient (Family 2) biopsies. Compared to control staining (left panel), TTC7A expression is reduced in the patient sample (right panel). Scale bar = 100 μm. (D) Stable knockdown of TTC7A resulted in morphological changes in Henle-407 cells. Expression of TTC7A in stably transfected Henle-407 cells was reduced (70%–80%) compared with Henle-407 cells stably transfected with control shRNA. The impact on cellular morphology of control and TTC7A shRNA knockdown was examined in Henle-407 cells by contrast microscopy (100× magnification) under normal culture conditions. Knockdown of TTC7A resulted in a loss of cobblestone morphology and development of fibroblastoid morphology with spindle-like features. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
mucosal epithelial degeneration. The intestinal phenotype observed in these 2 mouse models is reminiscent of the phenotype seen in our infantile patients who had massive shedding of enterocytes with increased apoptosis; however, none of the patients developed psoriasis or other skin abnormalities like the fsn mice. TTC7A has been investigated in human psoriasis and found not to be associated with human dermatological disease. Therefore, psoriasis might only be observed in the fsn mice and might not be part of the human disorder.

We have shown that TTC7A is expressed in enterocytes and has a role in enterocyte survival and function, suggesting that the physiological abnormalities observed in both mice and humans with TTC7A mutations result, at least in part, from epithelial dysfunction. However, as Chen et al also demonstrated, TTC7A is expressed in the thymus with a marked reduction of thymocytes and lymphoid depletion in 1 patient with TTC7A deficiency, TTC7A plays a critical role in modulating immune homeostasis and the immunodeficiency also contributes to the pathogenesis of TTC7A...
Figure 6. Summary of TTC7 mutations. Schematic representation of the role of TTC7A in the trafficking of PI4KIIIα to the plasma membrane from the trans-Golgi network. The left panel represents WT TTC7A in enterocytes wherein TTC7A binds to and facilitates the transport of PI4KIIIα from the trans-Golgi to the plasma membrane. At the membrane, PI4KIIIα can catalyze the production of PtdIns-4P(PI-4P). PI-4P levels at the plasma membrane have been implicated in cell survival and the maintenance of cell polarity. In the right panel, the various TTC7A mutations identified in the patients are depicted. E71K, Q526X, and A832T TTC7A all demonstrated reduced binding to PI4KIIIα, which could reduce the interaction between TTC7A and PI4KIIIα, hindering transport to the plasma membrane (PM). Consequently, this will lead to reduced plasma membrane levels of PI-4P, a dysregulation that would affect downstream signaling pathways.

Figure 5. (A) Tandem mass spectrometry. E71K and Q526X mutations reduce the ability of TTC7A to immunoprecipitate PI4KA. Selected peptides from PI4KA and TTC7A were analyzed to determine the area under their MS1 peaks to assess the relative abundance of each peptide. The PI4KA present in each sample was normalized to the total TTC7A in each technical replicate to allow comparisons among biological replicates (n = 3). These normalized values were averaged over all experiments. Error bars represent the standard error. (B) PI4KIIIα-TTC7A co-immunoprecipitate. HEK293T cells were transiently transfected with Myc-tagged WT (WT-TTC7A), E71K, Q526X, and A832T TTC7A constructs. Lysates were immunoprecipitated with anti-Myc antibody, and then immunoblotted using anti-PI4KIIIα and anti-Myc (for TTC7A) antibodies. The control lane represents transfection with an empty vector. (C) Expression and localization of PI4KIIIα is altered in patients with TTC7A deficiency. Immunofluorescence microscopy was performed on both control and patient colonic tissue sections immunostained with anti-PI4KIIIα antibodies. In the left panel, immunohistochemistry demonstrated that PI4KIIIα is highly expressed in enterocytes and immune cells from healthy human intestine. Inset panel depicts zoomed view of left panel, demonstrating PI4KIIIα expression at the plasma membrane of enterocytes. In the patient tissues, immunohistochemistry demonstrated that PI4KIIIα is dysregulated in enterocytes. Inset panel (representing region indicated by white arrow) demonstrates loss of PI4KIIIα at the plasma membrane of enterocytes bordering the intestinal crypt. Scale bar = 100 μm. (D) shRNA-mediated knockdown of TTC7A expression leads to decreased PI4KIIIα levels. To test the efficacy of the TTC7A shRNA, Henle-407 cells were transiently co-transfected with WT TTC7A and the various knock-down constructs, labeled #1 through #4, including a scrambled shRNA control and sham transfection. shRNA #1 and #3 showed reduction in TTC7A expression (left panels). shRNA containing the same targeting sequences were used to lentivirally infect Henle-407 cells where expression of PI4KIIIα was assessed in cell lysates by Western blot (right panels). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was stained as loading control for all blots. The intensity of each PI4KIIIα band was normalized to the GAPDH loading control by densitometry. Quantitation of band intensities (listed below each lane) demonstrates a statistically significant reduction in PI4KIIIα expression after TTC7A knockdown (Student t test, n = 3, P = .0234). Each normalized band intensity is presented as ±SEM. (E) TTC7A depletion results in decreased cytoplasmic phosphatidylinositol 4-phosphate (PI-4P) production. TTC7A knockdown and control Henle-407 cells were stained with antibodies against PtdIns4P (in red; Z-P004, IgM, Cedarlane, Burlington, NC) and 4',6-diamidino-2-phenylindole (DAPI) (in blue) to visualize nuclei. TTC7A knockdown Henle-407 cells have reduced plasma membrane immunostaining for PI-4P compared with controls. For control and TTC7A knockdown (KD), Henle-407 cells Z-stack images were generated at 0.2-mm intervals and recapitulated using Volocity to generate a 3-dimensional model to illustrate cell surface levels of PIP. Unconjugated green fluorescent protein, expressed from the control and knockdown plasmids, was visualized and used to approximate the morphology of the cells. Each pair of images represents 2 views of the same cell according to axes depicted.
deficiency, as seen in Family 1 and Family 2. These results are consistent with those seen in the MIA patients described with SCID11,12,33 and points to a severe defect in both enterocyte and T-cell function; however, patients from Family 3 did not have an overt T-cell defect and patients with MIA described previously11,12,33 had varying degrees of immunodeficiency, with some patients exhibiting mild T-cell lymphopenia in Chen et al,13 who also suggested an enterocyte defect based on the high frequency of bloodstream infections with intestinal microbes.

Therefore, our studies also suggest that mutations in the TTC7A gene can result in a spectrum of intestinal disease ranging from VEOIBD with apoptotic enterocolitis, as first described here, to MIA with SCID, as described here and previously.11,12,33 In support of this, TTC7A mutations were found to cause hereditary MIA with SCID11,12,33; however, apoptotic enterocolitis has not been reported. The patients from Family 1 and Family 3, with apoptotic enterocolitis with no evidence of MIA or structuring disease on autopsy, had mutations that would be predicted to reduce TTC7A expression but not completely abolish function. In support, we also demonstrated that the mutations identified in Family 1 and Family 3 reduced TTC7A binding to PI4KIIIα. Therefore, it is possible that the disease observed in patients from Family 1 and Family 3 represents a hypomorphic state, where some residual TTC7A activity in both enterocytes and the thymus results in severe enterocolitis without MIA and with or without lymphopenia.

All TTC7A-deficiency patients, including the patients described here, died in infancy due to their progressive bowel disease, failed allogeneic hematopoietic stem cell transplantation, or survived with short gut and total parenteral nutrition.11,12 Interestingly, both Chen et al11 and Samuels et al12 described an MIA TTC7A-deficiency patient who had hematopoietic stem cell transplantation and developed severe recurrence of MIA post transplantation. The recurrence of MIA after resection of atretic regions, and the enterocyte defect in both enterocytes and the thymus results in severe enterocolitis without MIA and with or without lymphopenia.

In Family 2, the TTC7A-deficiency patient, patients described here, died in infancy due to their progressive bowel disease, failed allogeneic hematopoietic stem cell transplantation, or survived with short gut and total parenteral nutrition.11,12 Interestingly, both Chen et al11 and Samuels et al12 described an MIA TTC7A-deficiency patient who had hematopoietic stem cell transplantation and developed severe recurrence of MIA post transplantation. The recurrence of MIA after resection of atretic regions, and the enterocyte defect in both enterocytes and the thymus results in severe enterocolitis without MIA and with or without lymphopenia.

Therefore, as we have demonstrated that an enterocyte defect is also found in patients with TTC7A deficiency, transplantation of allogeneic hematopoietic stem cells might not be warranted in TTC7A-deficient patients. However, our study opens the possibility of pharmacologically targeting the PI4KIIIα−TTC7A−Efr3B pathway as a potential therapeutic approach. The identification of TTC7A as a candidate gene for a unique and unrecognized variant of severe apoptotic enterocolitis expands the genetic diversity of VEOIBD and the need to tailor therapeutic approaches for individual subtypes.

Supplementary Material
Note: To access the supplementary material accompanying this article, visit the online version of Gastroenterology at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2014.01.015.


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The authors disclose no conflicts.

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