

# **TTC7A: Steward of Intestinal Health**



Sasha Jardine,<sup>1,2</sup> Neel Dhingani,<sup>1,2</sup> and Aleixo M. Muise<sup>1,2</sup>

<sup>1</sup>SickKids Inflammatory Bowel Disease Center and Cell Biology Program, Research Institute, <sup>2</sup>Division of Gastroenterology, Hepatology, and Nutrition, Department of Pediatrics, Institute for Medical Science and Biochemistry, University of Toronto, Hospital for Sick Children, Toronto, Ontario, Canada



#### **SUMMARY**

This review discusses the progress and challenges in tetratricopeptide repeat domain 7A deficiency, a monogenic cause of severe intestinal disease and immunodeficiency. Patient reports and functional studies to date are amalgamated into graphic summaries, highlighting patient mutations and the known role of tetratricopeptide repeat domain 7A in the cell.

The increasing incidence of pediatric inflammatory bowel disease, coupled with the efficiency of whole-exome sequencing, has led to the identification of tetratricopeptide repeat domain 7A (TTC7A) as a steward of intestinal health. TTC7A deficiency is an autosomal-recessively inherited disease. In the 5 years since the original description, more than 50 patients with more than 20 distinct disease-causing TTC7A mutations have been identified. Patients show heterogenous intestinal and immunologic disease manifestations, including but not limited to multiple intestinal atresias, very early onset inflammatory bowel disease, loss of intestinal architecture, apoptotic enterocolitis, combined immunodeficiency, and various extraintestinal features related to the skin and/or hair. The focus of this review is to highlight trends in patient phenotypes and to consolidate functional data related to the role of TTC7A in maintaining intestinal homeostasis. TTC7A deficiency is fatal in approximately two thirds of patients, and, as more patients continue to be discovered, elucidating the comprehensive role of TTC7A could show druggable targets that may benefit the growing cohort of individuals suffering from inflammatory bowel disease. (Cell Mol Gastroenterol Hepatol 2019;7:555–570; https:// doi.org/10.1016/j.jcmgh.2018.12.001)

*Keywords:* Inflammatory Bowel Disease; Monogenic; Very Early Onset IBD; Genetics; Whole-Exome Sequencing; Multiple Intestinal Atresia; PI4K; Primary Immunodeficiency.

**R** are autosomal-recessive variants in tetratricopeptide repeat domain 7A (*TTC7A*) have been discovered in patients presenting with severe intestinal and immune diseases.<sup>1-16</sup> Mounting evidence has indicated that *TTC7A* mutations can result in various phenotypes that may or may not be associated with combined immunodefi-

Abbreviations used in this paper: CID, combined immunodeficiency; EFR3, Protein EFR3 (EFR3); HSCT, hematopoietic stem cell transplantation; MIA, multiple intestinal atresia; P4KIIIalpha, phosphatidylinositol 4-kinase III alpha; PI4P, phosphatidyl inositol 4 phosphate; PIP, phosphatidyl inositol-phosphate lipid; ROCK, RhoA kinase; TPR, tetratricopeptide repeat; TTC7A, tetratricopeptide repeat domain 7A; TTC7B, tetratricopeptide repeat domain 7B.

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© 2019 The Authors. Published by Elsevier Inc. on behalf of the AGA Institute. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). 2352-345X https://doi.org/10.1016/j.jcmgh.2018.12.001 ciency (CID), including multiple intestinal atresias (MIA) and very early onset inflammatory bowel disease (VEOIBD), a form of IBD in children younger than 6 years of age.<sup>3,5,13</sup> Since 2013, there have been more than 50 TTC7A cases reported in the literature, and, unfortunately, more than half of these children died of the disease, with a median survival age of younger than 12 months.<sup>10</sup> Hematopoietic stem cell transplantation (HSCT) has been reported to recover immunologic defects; however, the intestinal disease was refractory to HSCT.<sup>10,11</sup> Multiple mutations in the TTC7A gene, combined with heterogenous disease presentation, has made it difficult to correlate phenotype and genotype.<sup>11</sup> TTC7A has 9 tetratricopeptide repeat (TPR) domains, a structural motif that is involved in multiprotein scaffolding.<sup>17</sup> Previous studies have confirmed that TTC7A acts as a scaffolding protein, where it binds and recruits phosphatidyl inositol 4 phosphatidylinositol 4-kinase III alpha to the plasma membrane, facilitating the synthesis of PI4phosphate (PI4P).<sup>3,18,19</sup> There is evidence to suggest that patients with immunodeficiency and homozygosity for mutations affecting the scaffolding TPR domains have a worse prognosis.<sup>11</sup> Furthermore, TTC7A mutations that are predicted to result in significant protein truncation and loss of function tend to result in more severe intestinal phenotypes (ie, MIA).<sup>1-3,5,7,9,13,14</sup> Alternatively, nontruncating mutations, termed hypomorphic variants, tend to present as VEOIBD.<sup>1-3,6,8,11,13</sup> Establishing an appropriate animal model to study TTC7A deficiency has been challenging, however, patient-derived intestinal organoids show defects in intestinal epithelial cell polarity.<sup>5,6,20</sup> Ultimately, the comprehensive structure, interactions, targets, and pathobiology of TTC7A remain to be elucidated, however, the focus of this review is to highlight the work that is being performed to understand TTC7A deficiency, from patient genetics to functional in vitro studies. Presently, as more patients are being discovered, there is a great need to unearth more about TTC7A so that target-specific therapies may be developed.

## TTC7A Biology

### A Conserved TPR-Domain–Containing Protein

TTC7A is a conserved gene and has homologs in the chimpanzee, mouse, chicken, zebrafish, and frog.<sup>21</sup> RNA expression of TTC7A is comparable in many tissues, making it unclear why patients with TTC7A mutations have phenotypes specifically related to gut and immune dysfunction.<sup>22</sup> Although there are 2 splice isoforms, the most common TTC7A splice variant has 20 exons and is 858 amino acids long.<sup>22</sup> TTC7A has a paralog, tetratricopeptide repeat domain 7B (TTC7B) with 49.47% sequence identity, and both (independently) are involved in the conserved PI4KIII $\alpha$  complex.<sup>18,19,23,24</sup> The early onset of TTC7A deficiency symptoms may hint at differences in the expression of TTC7A and TTC7B during embryonic development. During development, TTC7B is expressed in the cerebral cortex and cerebellum, whereas TTC7A is expressed in a wide range of tissues/organs, including several tissues of the brain, bone marrow, testis, pancreas, ovaries, liver, and blood.<sup>25,26</sup> In adults, TTC7B is expressed ubiquitously in the body, with higher expression in the brain, fat, and small bowel. TTC7B may have some redundancy with TTC7A because the small bowel is less affected in TTC7A patients.<sup>3,6,24</sup>

TTC7 proteins contain TPR domains, which are helixturn-helix structurally conserved motifs, thought to have roles in protein scaffolding.<sup>21</sup> The highly conserved secondary and tertiary structures of TPR domains have large surface areas that accommodate multiple-protein interactions.<sup>17,27</sup> Although the complete structure of TTC7A has yet to be elucidated, Baskin et al<sup>19</sup> resolved the crystal structure of TTC7B and confirmed the presence of large concave and convex surfaces formed by multiple TPR domains. TTC7B has a very large concave interface (approximately 600 nm, where anything upward of 200 nm is thought to be large) that can facilitate binding with PI4KIII $\alpha$ , FAM126A, and EFR3, members of the PI4KIII $\alpha$  complex.<sup>19,24</sup> TTC7A mutations affecting TPR domains tend to result in worse patient phenotypes and poor outcomes, implying that TPR-domain structure is critical to TTC7A's scaffolding function.<sup>11</sup> Interestingly, trichohepatoenteric syndrome, a severe condition defined by intractable infant diarrhea and immune abnormalities, is caused by defects in TTC37, another TPR-domain-containing protein.<sup>13,28</sup> Similar to TTC7A, little is known of the protein encoded by TTC37 and how mutations in both of these TPRdomain-containing proteins contribute to immune and gut dysfunction. Resolving TTC7A's comprehensive structure may further our understanding of how TPR domains contribute to the protein's molecular biology in the cell.

## A Scaffolding Protein for the PI4KIIIα Complex

Ten years ago, Baird et al<sup>29</sup> identified the yeast ortholog of TTC7A (Ypp1) and Efr3 as novel components of the PI4KIII $\alpha$  (Stt4) complex. Avitzur et al<sup>3</sup> subsequently showed that PI4KIII $\alpha$  is a specific TTC7A binding partner in mammalian cells via co-immunoprecipitation experiments. Tandem mass spectrometry showed that spectral hit levels of PI4KIII $\alpha$  are reduced significantly in TTC7A hypomorphic (having partial function) mutants, relative to hit counts with wild-type TTC7A.<sup>3</sup> Several studies have corroborated the role of TTC7 in recruiting and targeting PI4KIII $\alpha$  to the plasma membrane.<sup>18,19,24</sup> The TTC7A-PI4K complex is stabilized by an adaptor protein, FAM126A, and once at the plasma membrane, tethered by EFR3B, a membrane-bound protein (Figure 1).<sup>18</sup> TTC7A is localized diffusely in the cytosol, however, when co-expressed with EFR3, it localizes to the plasma membrane, further indicating a scaffolding role for PI4KIII $\alpha$  to Protein EFR3 (EFR3).<sup>18</sup> Formation of the PI4KIII $\alpha$  complex results in the phosphorylation of PI membrane lipids to form PI4P (Figure 1), which indicates that TTC7A has an upstream role in mediating PI4P synthesis.<sup>18,19,24</sup> The effect of TTC7A on PI4P levels has been shown via TTC7A knockdown experiments that show a decrease in the immunostaining for PI4P membrane lipids.<sup>3</sup> Plasma membrane PI4P synthesis is dependent exclusively on the proper functioning of PI4KIII $\alpha$ , and homeostatic levels of PI4Ps are important for plasma membrane identity, polarity, cell survival, and production of phosphatidylinositol 4,5-bisphosphate (PI[4,5]P<sub>2</sub>)/phosphatidylinositol 3,4,5trisphosphate (PI[3,4,5]P<sub>3</sub>].<sup>30</sup> In mice, transient loss of PI4KIII $\alpha$  in fibroblasts results in cell death, and germline loss results in embryonic lethality, indicating that PI4KIII $\alpha$ has a critical role in survival pathways.<sup>18</sup> The link between PI4KIIIα, intestinal integrity, and overall viability was shown elegantly with mice harboring an inducible PI4KIII $\alpha$ knockout mutation that caused fatality and gastrointestinal tract lesions, similar to TTC7A-deficiency patient defects.<sup>31</sup> Specifically, the conditional knockout of PI4KIII $\alpha$  in mice resulted in gastrointestinal distension (owing to the loss of intestinal emptying), necrosis, and extensive mucosal destruction, while other tissues (ie, brain, heart, and lungs) were unaffected, indicating an essential role for PI4KIII $\alpha$  in intestinal health.<sup>31</sup>

Cryo-electron microscopy recently showed that the PI4KIII $\alpha$  complex can dimerize to form a large and stable "superassembly."<sup>24</sup> Two PI4KIII $\alpha$  enzymes form a homodimer, in which the dimerization interface is held at 2 contact positions by TTC7, suggesting that TTC7 plays an important role in stabilizing the large PI4KIII $\alpha$ -heterodimer complex.<sup>24</sup> Co-immunoprecipitation experiments have shown that the formation of the PI4KIII $\alpha$  complex is possible with hypomorphic TTC7A variants, however, minor alterations to the TTC7A-PI4KIII $\alpha$  assembly nonetheless may affect kinase activity.<sup>3</sup> Specifically, mutations in the C-terminus of TTC7A and N-terminus of PI4KIII $\alpha$  result in PI4KIII $\alpha$ -complex formation, whereas kinase activity is lost.<sup>24</sup> The PI4KIII $\alpha$  complex is conserved from yeast<sup>32</sup> to human beings, where PI4KIII $\alpha$  interaction with TTC7 and FAM126 restricts its conformation so that its kinase domain can interact with PIs on the plasma membrane.<sup>24</sup> The activity and localization of the PI4KIII $\alpha$  complex depends on several contact sites between the FAM126-TTC7A-PI4KIIIα-EFR3B proteins and the plasma membrane, providing grounds for how hypomorphic TTC7A mutations may have critical functional consequences for the tightly regulated PI4KIII $\alpha$  complex.<sup>18,19,24</sup>

Although the PI4KIII $\alpha$  complex is responsible for PI4P synthesis at the plasma membrane, it is important to recognize the plasticity of PI4P levels and the dynamic nature of phosphatidyl inositol-phosphate lipids (PIPs) throughout the cell.<sup>30</sup> Despite insults to the PI4KIII $\alpha$  complex, PI4Ps still can be present at the plasma membrane owing to several compensatory pathways, including but not limited to: other subcellular membranes with PI4Ps can fuse with the plasma membrane, TMEM150A (a membranebound protein) stabilizes PI4KIII $\alpha$  at the membrane in the absence of TTC7A, TTC7B redundancy, and other PIP kinases and phosphatases potentially replenishing pools of various plasma membrane PIPs.<sup>18,19,24,30</sup> PI4P synthesis represents a critical regulatory threshold in the plasma membrane PI pathway and an important mediator of cellular homeostasis.<sup>30</sup> PI4P and its role as a precursor in the PI pathway is partly dependent on a functional TTC7A, which is involved in tethering, shuttling, and stabilizing the conserved PI4KIII $\alpha$  complex. A thorough understanding of the pathobiology related to PI4P levels in TTC7A deficiency has yet to be elucidated.

## Increased Apoptosis and Loss of Polarity: Phenotypic Insights From Functional Studies

Immunohistochemical staining of healthy intestinal biopsy specimens has confirmed that TTC7A is expressed in intestinal cells and localizes intracellularly at the plasma membrane.<sup>3</sup> Staining of patient biopsy samples with truncating *TTC7A* mutations show a total loss of protein, suggesting nonsense-mediated decay of TTC7A messenger RNA transcripts.<sup>1,3</sup> The absence of TTC7A in immunohistochemical staining also is consistent with the increased phenotypic severity in patients with MIA-CID, further associating protein truncations with complete loss of function (Figure 2).<sup>1–5,7,9–11</sup>

TTC7A knockdown in intestinal Henle-407 cells with short hairpin RNA disrupts their characteristic cobblestone morphology and impairs adhesion to collagen/fibronectin.<sup>3</sup> These features are in line with endoscopic observations from patients showing loss of epithelial integrity and mucosal sloughing.<sup>3,5,6,11</sup> TTC7A deficiency also results in increased caspase 3 cleavage, which is a cysteine–aspartic acid protease that is important in mediating apoptosis once cleaved.<sup>3,5</sup> The findings show that TTC7A deficiency results in increased susceptibility to apoptosis, a phenotype consistent with patient histology showing higher intestinal epithelial cell apoptosis.<sup>3,5,6,8</sup>

The polarized monolayer of intestinal epithelial cells is an elegant example of form and function because it creates a barrier that protects against inappropriate immune responses and facilitates nutrient absorption. In TTC7A deficiency, stenosis can arise from alterations to the integrity of the intestinal epithelium.<sup>33,34</sup> Intestinal epithelial cell polarity, for instance, normally restricts epithelial cell growth to a monolayer, however, when highly organized epithelial cells lose an aspect of their polarity, the epithelium may become stratified or pseudostratified, characteristics associated with intestinal pathologies.<sup>33,34</sup> Bigorgne et al<sup>5</sup> showed that TTC7A dysfunction results in aberrant intestinal epithelial cell polarity. Ileum-derived intestinal organoids (or enteroids) were cultured from a patient with homozygous p. A832fsX1 mutations that disrupt the final exon, however, it is unknown whether TTC7A is hypomorphic, or absent, because no expression levels were provided.<sup>5</sup> The organoid donor was the only surviving member of the study cohort and presented with intestinal atresias from birth, lymphocytopenia, hypogammaglobulinemia, and progressive skin conditions from 4 years of age.<sup>5</sup> The organoids were stained for intestinal epithelial cell polarity markers, filamentous actin and  $\alpha 6$  integrin, which designate apical and basal membranes, respectively.<sup>5</sup> The TTC7A patient-derived organoids showed several abnormalities: filamentous actin staining on the basal membrane, an illdefined luminal space, a disorganized pattern of  $\alpha 6$  integrin, significantly reduced proliferation, dense aggregates with no obvious luminal space, and reduced budding.<sup>5</sup> Taken together, TTC7A-deficient organoids showed disrupted apicobasal polarity and poor epithelial cell integrity.

Remarkably, exposure of the TTC7A patient organoids to a RhoA kinase (ROCK) inhibitor (10  $\mu$ mol/L Y-27632 for 5

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days) corrected the polarity defect and resulted in increased proliferation.<sup>5</sup> Apicobasal polarity is partly regulated by the RhoA pathway via cytoskeletal proteins, where down-stream effectors (such as myosin light chain and ezrin-radixin-moesin) are important in filamentous actin dynamics, stress fiber contractility, and adhesion (ie, tight junctions, integrins).<sup>5,35</sup> Higher protein expression of the downstream targets of ROCK were uncovered in these organoids, and subsequently reduced with ROCK-inhibitor treatment.<sup>5</sup> Thus, the data infer that TTC7A, through an unknown mechanism, acts to regulate cytoskeletal dynamics via myosin light-chain activation (Figure 1). Alternatively, ROCK inhibition may be acting on a TTC7A-independent pathway. It is intriguing to suggest that ROCK activity may mediate intestinal epithelial cell polarity, although the exact



mechanism by which TTC7A is involved in the RhoA/ROCK pathway is unknown.<sup>35,36</sup>

In contrast to the intestinal epithelial cell phenotypes associated with TTC7A dysfunction, Lemoine et al<sup>6</sup> found that lymphocytes lacking TTC7A have increased proliferation, adhesion, and migration, all of which were reduced with ROCK-inhibitor treatment. The data suggest that TTC7A also regulates the actin cytoskeleton in lymphocytes. Increases in the activation of effectors downstream of ROCK in lymphocytes and intestinal epithelial cells seem to disrupt normal cell homeostasis and epithelial-immune dynamics contributing to inflammation.<sup>6,37</sup> Elucidating the cellspecific nuances of TTC7A deficiency may provide insight into how therapies could have differential effects on epithelial and immune cells.



# Underlying Genetics of TTC7A Deficiency

The role of genetics in the development of IBD has been well established via genome-wide association studies, which have identified more than 200 IBD susceptibility loci contributing to polygenic or typical IBD.<sup>38</sup> Genome-wide association studies, a statistically based genetic analysis, is blind to rare variants, especially those that cause severe disease and increased mortality, which further dilutes these rare alleles from the population's gene pool.<sup>39,40</sup> In contrast, whole-exome sequencing often is used for diagnosis when single genetic mutations are suspected to be causative. For example, the majority of known rare diseases are attributed to defects in a single gene, as opposed to being driven by multiple variants or an unknown etiology.<sup>41</sup> Similarly, more than 50 genes have been uncovered that ascribe monogenic IBD-like phenotypes.<sup>42</sup> Of those genes, single variants in TTC7A are shown to cause intestinal and immune disease with high penetrance.<sup>1–11,13,14,16</sup> Recently, Lien et al<sup>11</sup> performed TTC7A genotype/phenotype comparisons via Kaplan-Meier analysis. They showed that patients with autoimmune defects and biallelic missense mutations (not affecting TPR domains) had better survival outcomes.<sup>11</sup> Ultimately, it remains difficult to correlate genotype with phenotype because there are more than 20 TTC7A mutations linked with heterogenous disease features (Table 1), which, in turn, are associated with varying morbidities and mortalities.<sup>1–11,13,14,16</sup>

## Lessons from Hypomorphic and Loss-of-Function TTC7A Variants

Missense mutations, resulting in the substitution of one amino acid for another, are thought to result in hypomorphic *TTC7A* variants, oftentimes presenting with clinical features reminiscent of monogenic IBD, or, more specifically, VEOIBD given the age of patients.<sup>1,3,5,6,8,11,13,43</sup> The MIA-CID phenotype is associated with greater morbidity and mortality and tends to be associated with complete loss of function because of truncating *TTC7A* mutations (ie, nonsense, frameshifts, large deletions).<sup>1–5,7,9–11</sup> For example, a patient compound heterozygous for missense (p. E71K) and truncation (p. Q526X) mutations showed no clinical or pathologic evidence of atresias.<sup>3</sup> Patients with mutations affecting splice acceptor sites, resulting in the skipping of exons and premature stop codons, have large peptide stretches missing from TTC7A and present with atresias, severe inflammation, and increased intestinal cell apoptosis.<sup>1</sup>

Analysis of patient outcomes (Figure 2) loosely correlate poor survival to truncating mutations, which are predicted to result in complete loss of TTC7A function because of transcript degradation via nonsense-mediated decay.<sup>2,3,6,7</sup> At odds with the prediction of nonsense-mediated decay, Yang et al<sup>9</sup> reported that a 45–amino acid in-frame deletion and a nonsense mutation in the final exon of TTC7A had similar reverse-transcription polymerase chain reaction amplifications to control DNA, suggesting that these transcripts were not subject to nonsense-mediated decay mechanisms. However, no protein expression data were provided for these patients, who ultimately died of their disease.<sup>9</sup> Furthermore, the French Canadian patients reported by Samuels et al<sup>1</sup> also had a large deletion causing the loss of exon 7 and similarly were found to have "sufficient" RNA levels, although no protein expression was reported. Interestingly, fibroblasts and B cells derived from TTC7A-deficiency patients harboring the same deletion presented in the Samuels et al<sup>1</sup> report showed that TTC7A protein expression is undetectable, suggesting nonsensemediated decay of messenger RNA transcripts.<sup>24</sup> As illustrated in Figure 2, all deceased TTC7A patients had at least 1

Figure 1. (See previous page). Summary of the role of TTC7A in intestinal epithelial cells and the consequences of its mutation. (A) TTC7A competent. TTC7A and FAM126A chaperone PI4KIII $\alpha$  to the plasma membrane.<sup>19</sup> EFR3 tethers the PI4KIII $\alpha$  complex to the plasma membrane where PI4KIII $\alpha$  catalyzes the conversion of PI to PI4P.<sup>18,19,24</sup> The synthesis of PI4P is an important regulatory step in the phosphatidyl inositol-phosphate pathway, which is important for survival pathways and intestinal epithelial cell polarity.<sup>30</sup> Phosphatidyl inositol-phosphate lipids are differentially produced and turned over on apical and basal membranes of polarized intestinal epithelial cells; thus, phosphatidyl inositol-phosphate levels aid in coordinating apicobasal polarity, which is critical for epithelium integrity.<sup>75</sup> Cytoskeletal homeostasis is important in maintaining cell polarity and cell adhesions.<sup>5,35</sup> Tight junctions, adherens junctions, and integrins are regulated in part by actin dynamics and maintain cell adhesions, contributing to intestinal epithelial barrier function.<sup>36,76</sup> (B) TTC7A deficiency. Truncating mutations (ie, nonsense, frameshifts, large deletions) are associated with more severe phenotypes and are predicted to cause a complete loss of function in which TTC7A transcripts are thought to be degraded via nonsense-mediated decay.<sup>2,3,6,7,24</sup> Because TTC7A is a scaffold for multiple proteins, its structure is critical to its role in binding, shuttling, and tethering the PI4KIIIa complex to the plasma membrane; thus, partial or total loss of TTC7A reduces PI4P synthesis (1) via the PI4KIII $\alpha$  complex (dotted arrow).<sup>3,19</sup> Alterations to the structure of TTC7A (ie, truncated or hypomorphic protein) have been shown to alter the kinase activity and/ or the positioning of PI4KIIIa at the membrane.<sup>24</sup> Decreases in PI4P affects cell polarity (2), signaling, homeostasis, and survival.<sup>30</sup> H&E staining of intestinal biopsy specimens from TTC7A-deficiency patients showed pseudostratification, which points to defects in apicobasal polarity (3). Similarly, tightly regulated apical and basal membranes, marked by the actin belt and integrins, respectively, were mislocalized in TTC7A patient-derived organoids.<sup>5,6</sup> Loss of apicobasal polarity jeopardizes the integrity of the epithelium, contributing to the diverse epithelial defects observed in TTC7A deficiency.<sup>5,6</sup> (4) An increase in intestinal epithelial cell apoptosis, activated via caspase cleavage, was a common feature in patient histology and in vitro functional studies.<sup>3,5,6,8</sup> TTC7A deficiency, through unknown mechanisms (dashed arrow), is correlated with increased phosphorylation of myosin light chain (5) and numerous other downstream targets in the RhoA pathway (omitted for simplicity).<sup>5</sup> Myosin light-chain phosphorylation initiates actomyosin contraction, which can disturb junction and integrin adhesion pro-teins and promote cell lifting (6).<sup>35,36,77,78</sup> Increases in intestinal epithelial cell apoptosis, actomyosin contraction, and cell lifting disrupts epithelial barrier function (7).<sup>54,56</sup> Furthermore, a weakened intestinal barrier results in the translocation of luminal bacteria into the lamina propria, which can trigger an inflammatory response (8).<sup>34,76</sup> The role of TTC7A in the cell is incompletely understood, making it difficult to link its known functions to the development of MIA, another poorly understood disease.



**Figure 2. Known TTC7A mutations grouped by patient outcomes.** Patient naming codes are derived from Table 1 and are shown in red or black to indicate deceased or living patients, respectively. Within patient outcome groupings (ie, deceased, living, or mixed), patients are arranged so that deletion, frameshift, or nonsense mutations affecting both alleles are listed first, followed by patients with missense mutations on at least 1 allele. TTC7A has 20 exons that include 9 TPR domains that are represented by the complementary DNA and protein schematics, respectively (NM\_020458.3 and NP\_065191.2). Unaffected protein sequences are indicated in dark grey, while *grey connecting lines* indicate exon deletions. Light grey stretches are downstream of frameshift and deletion mutations and represent areas where there is a predicted loss of protein expression resulting from nonsense-mediated decay mechanisms.<sup>2,3,6,7,24</sup> Specific amino acid mutations are indicated by color bars: blue, missense; red, nonsense (ie, stop codon or X); green, frameshifts. Patient M1 is not represented because of a lack of specifics regarding their deletion mutation. ER, endoplasmic reticulum; NMD, nonsense mediated decay.

Table 1. Mutations, Clinical Phenotypes, and Outcomes of Reported TTC7A-Deficiency Patients											
Patient	Cited mutations <sup>a</sup>	TPR $\Delta$	Ethnicity	MIA	IBD	CID	Alive/deceased, age <sup>b</sup> ; cause				
S1-5 <sup>1</sup>	Hom c.1000+ 3del AAGT, Ex7 del	+	French Canadian	+			Deceased, <7 days; MIA				
S6 <sup>1</sup>	Het c.1000+ 3del AAGT, Ex7 del; c. 133074 A>G Ex20; p.L823P	+	French Canadian	+			Deceased, 47 days; MIA				
S7 <sup>1</sup>	Het c.1000+ 3del AAGT, Ex7 del; c. 133074 A>G Ex20; p.L823P	+	French Canadian	+	+	+	Deceased, 1 y; MIA				
C1 <sup>2</sup>	Hom c.1919+1G>A Ex16 del	+	Arabic	+		+	Deceased, 3 mo; Candida sepsis				
C2 <sup>2</sup>	Hom c. 313 del TATC, Ex2/Ex3 del p.Y105fs	+	Serbian	+		+	Deceased, 1 mo; MIA				
C3 <sup>2</sup>	Hom c. 313 del TATC, Ex2/Ex3 del p.Y105fs	+	Bosnian	+		+	Alive, 2.8 y				
C4 <sup>2</sup>	Het c.762 del G Ex5;p.K254fs c.2468T>C Ex20; p.L823P	+	Unknown	+		+	Alive, 2 y				
C5 <sup>2</sup>	Het c.1817A>G Ex16, p.K606R $^\circ$ c.2014 T>C Ex17, p.S672P $^\circ$ c.1000+ 3del AAGT, Ex7 del		French Canadian, mixed European	+		+	Alive, 22 mo				
C6 <sup>2</sup>	Het c.2033C>A Ex18; p.S678X; c.2134C>T Ex18; p.Q712X	+	Italian	+	+	+	Deceased, 10 mo; pneumonitis				
C7 <sup>2</sup>	Hom c.1196T>C Ex9; p.L399P		Italian	+		+	Alive, 9 mo				
C8 <sup>2</sup>	Unknown mutations Ex2 Ex3 del	+	Italian	+		+	Deceased, 8 mo; sepsis				
A1 <sup>3</sup>	Het c.211G>A Ex2; p.E71K; c.1578C>T Ex14; p.Q526X	+	Caucasian/Sudanese	+	+	+	Deceased, 11 mo; parainfluenza pneumonia, ARDS				
A2 <sup>3</sup>	Het c.844-1G>T, Ex 7 del; c.1204-2A>G, Ex 10 del	+	Caucasian	+	+	+	Deceased, 3 mo; pulmonary embolism				
A3 <sup>3</sup>	Het c.844-1G>T, Ex 7 del; c.1204-2A>G, Ex 10 del	+	Caucasian	+		+	Deceased, 19 mo; cardiac arrest				
A4 <sup>3</sup>	Hom c.2494 G>A Ex20; p.A832T	+	Caucasian		+		Alive, 14 mo				
A5 <sup>3</sup>	Hom c.2494 G>A Ex20; p.A832T	+	Caucasian		+		Deceased, 11 mo; Candida sepsis				
Ag1 <sup>4</sup>	Het c.1652 C>A Ex15; p.A551D c.2482C>T Ex20; p.E828X	+	Irish/Ashkenazi Jewish	+		+	Alive, 7 mo				
B1 <sup>5</sup>	Het c.185-348 del Ex2; p.D62-S116 del c.185-517 del Ex2/3; p.D62-173G del		Mixed European	+		+	Deceased, 2.5 y; sepsis				
B2 <sup>5</sup>	Hom c. 829 C>T; p.Q277X Ex 6 del		Saudi Arabian	+		+	Deceased, 9 mo; pulmonary hemorrhage				
B3 <sup>5</sup>	Hom c.2496 del CG Ex20, p.A832X	+	Sri Lankan	+		+	Alive, 73 mo				
B4 <sup>5</sup>	Het c.1288-1392 del Ex 12 del; c.1616C>T Ex 14; p.S539L		Norwegian	+		+	Deceased, 8 mo; sepsis				
B5 <sup>5</sup>	Het c.1008C>G Ex8; p.Y336X c.1479 delG Ex 12; p.L493 fsX13		Mixed European	+		+	Deceased, 3.9 y; virus pneumonia				
B6 <sup>5</sup>	Het c.1510+105 T>A Ex12 del; c.1673 insG Ex15; p.A558GfsX7		Mixed European	+		+	Deceased, 7 mo; cytomegalovirus pneumonitis				
$L1^{\circ}$ $L2^{6}$ $L5^{6}$ $L7^{6}$ $L7^{6}$ $L8^{6}$ $L9^{6}$ $L10^{6}$ $L11^{6}$ $L12^{6}$ $L13^{6}$	Hom c.211G>A Ex2, p.E71K Hom c.211G>A Ex2, p.E71K		French		+	+	Alive, 5 mo Deceased, 6 mo; HSCT Deceased, 9 mo; HSCT Deceased, 8 mo; enteropathy Alive, 10 mo Alive, 10 mo Alive, 2 y Alive, 4 y Deceased, 4 y; sepsis Deceased, 14 y; gastric carcinoma Alive, 14 y Alive, 28 y Alive, 50 y				

Table 1. Continued									
Patient	Cited mutations <sup>a</sup>	TPR $\Delta$	Ethnicity	MIA	IBD	CID	Alive/deceased, age <sup>b</sup> ; cause		
L14 <sup>6</sup>	Het c.911T Ex7; p.L304 fsX59; c.1433T>C Ex12, p. L478P		French		+		Alive, 18 mo		
G1 <sup>7</sup>	Hom c. 313 del TATC, Ex2/Ex3 del p.Y105fs	+	Bosnian	+		+	Alive, 3 y		
W1 <sup>8</sup>	Hom c.1037 T>C Ex8; p.L346P		Turkish		+	+	Deceased, 15 mo, sepsis		
Y1 <sup>9</sup>	Het c.2018-2 A>G; Ex18 del c.2569 G>T Ex20; p.E857X	+	Malaysian	+			Deceased, 27 mo, sepsis		
Y2 <sup>9</sup>	Het c.2018-2 A>G; Ex18 del c.2569G>T Ex20; p.E857X	+	Malaysian	+			Deceased, 3 days, sepsis		
K1 <sup>10</sup>	Het p.E191FsX; Ex4 p.I854F Ex20	+	Mediterranean	+			Alive, 77 mo		
K2 <sup>10</sup>	Hom p.G45_A55 del Ex1		Middle Eastern	+		+	Alive, 86 mo		
K3 <sup>10</sup>	Het p.E71K Ex2 p.E96 Ex2		British	+		+	Alive, 20 mo		
Li1 <sup>11</sup>	Het c.223 G>A p.E75K Ex2 c. 520-521 del CT fs174X27 Ex4	+	Taiwanese	+	+		Deceased, 8 mo, sepsis		
Li2 <sup>11</sup>	Het c.223 G>A p. E75K Ex 2 c. 520-521 del CT fs174X27 Ex4	+	Taiwanese	+	+		Deceased, 4 mo, liver failure		
La1 <sup>12</sup>	Het c.1817A>G; p.K606R Ex16 c.2014 T>C; p.S672P Ex17		European			CVID	Alive, 15 y		
N1 <sup>13</sup>	Het c.1616 C>T p. S539L Ex14 c. 2515 G>A p.A839T Ex20	+ +	Portuguese	+	+	+	Alive, 3 y		
M1 <sup>14</sup>	Hom c. 53344_53347 del		Unknown Consanguineous	+		+	Deceased, 8 mo; graft-versus-host disease, liver failure		
F1 <sup>15</sup>	Hom c.1709A>G; p.H570R Ex15	+	North African	+	+	+	Alive, 43 mo		
F2 <sup>15</sup>	Het c. 189C>G; p.D63E Ex2 c. 412 C>T; p.R138X Ex3	+	Unknown		+	+	Alive, 18 mo		

NOTE. Superscript numbers in Patient column refer to reference citations.

ARDS, acute respiratory distress syndrome; c, complementary DNA location; CVID, common variable immune deficiency; Het, compound heterozygous mutation; Hom, homozygous mutation; p. protein or amino acid location.

<sup>a</sup>National Center for Biotechnology Information reference sequences for TTC7A were absent or varied between publications. Among the 15 reports citing TTC7Adeficiency patients, 6<sup>3,5,6,8,9,15</sup> referenced mutations with NM\_020458.3 (transcript variant 2, coding sequence from 369 to 2945 nucleotides) and NP\_065191.2 (isoform 2, 858 amino acids). Lien et al<sup>11</sup> and Neves et al<sup>13</sup> reported mutations based on NM\_001288951.1 (transcript variant 1, coding sequence from 369 to 3017 nucleotides) and NP\_001275880.1 (isoform 1, 882 amino acids). Samuels et al<sup>1</sup> based DNA annotations on NC\_000002.11 because one of the mutations affected an intronic region, however, the protein annotation was based on NP\_065191.2. To our knowledge, the remaining 6<sup>2,4,7,10,12,14</sup> publications did not report the National Center for Biotechnology Information coding sequence and protein reference codes.

Surviving patient age is as reported in the original publication.

<sup>°</sup>Maternal.

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allele affected by deletion, nonsense, or frameshift mutations. Approximately half of this group had truncating or deleterious mutations on both alleles, providing a rationale for the poor survival outcomes of these patients and suggesting a total loss of TTC7A function.

Despite the previous examples, we are not yet able to draw any hard and fast rules regarding correlations between patient genotypes and phenotypes. TTC7A deficiency seems to result in a range of complex phenotypes not easily attributed to physical alternations on the *TTC7A* gene. For example, Woutsas et al<sup>8</sup> reported a patient with MIA-CID who was homozygous for a substitution mutation at a conserved site (p. L346P). HEK293T cells expressing the p. L346P mutation confirmed that the variant had expression levels that were stable and similar to wild-type TTC7A levels, making this a hypomorphic variant, yet the patient presented with the MIA phenotype.<sup>8</sup> These findings highlight the discrepancies between genotype and phenotype in TTC7A patients and indicate that the hypomorphic/ truncated dichotomous framework may be incomplete.

To understand the effects of specific TTC7A mutations that contribute to complete loss of protein function, more protein analysis via immunostaining or immunoblotting is required. For example, protein analysis was limited among the 15 publications discussed here: 3 provided TTC7A immunohistochemical staining of the thymus,<sup>2</sup> small bowel,<sup>12</sup> and cecum,<sup>3</sup> and 2 provided immunoblot protein analysis from TTC7A mutant overexpression cell lines.<sup>3,8</sup> To fully appreciate the pathogenicity of specific *TTC7A* mutations, site-specific and knock-in mutagenesis studies in disease models will aid in understanding the relationship between genetics and disease phenotypes.

# Clinical Presentation of TTC7A Deficiency

Advancements in diagnostic genetic screening has uncovered mutations in the TTC7A gene that manifest as severe intestinal disease (the focus of this review) and primary immunodeficiency in pediatric patients.<sup>1–11,13,14,16</sup> Young children (age, <2 y) experiencing aggressive and refractory intestinal disease and with a family history of immunologic and/or intestinal disease are candidates for genetic testing and diagnosis.44 Identification of an underlying monogenic cause of disease is typically a collaborative effort involving the patient, their family, clinical teams (ie, endoscopy, biopsy), and laboratory testing (ie, histology, blood processing, candidate gene screening, and identification).<sup>42</sup> Although many TTC7A-deficiency reports have identified mutations that are suspected to drive the disease, few studies have performed functional analysis to confirm the phenotype and pathogenicity of specific variants. Within 5 years, there have been 55 TTC7A cases described in 15 reports (Table 1), and, unfortunately, many of these patients died of their disease with a median survival age younger than 12 months.<sup>10</sup>

#### MIAs With or Without CID

In 2013, a French Canadian cohort of patients with hereditary MIA, a severe and fatal form of congenital bowel

obstruction, were found to have TTC7A deficiency.<sup>1</sup> MIA presents as discontinuity of the intestinal lumen caused by obstructions found anywhere along the pylorus to the rectum, and despite surgical resections the obstructions tend to be recurrent.<sup>45</sup> Intestinal atresias are estimated to occur in 1 in 5000 newborns, in whom jejunoileal atresias are most prevalent.<sup>46</sup> Idiopathic intestinal obstruction and stenosis have been reported in IBD, severe CID, and graftversus-host disease, conditions involving aberrant inflammatory responses. In particular, obstructions are relatively infrequent in ulcerative colitis, yet are observed in the small bowel (25% of cases) and colon (10% of cases) in patients with Crohn's disease.<sup>46,47</sup> TTC7A-deficiency patients have been reported to have widespread atresias affecting the pylorus, small bowel, appendix, ileocecal valve, colon, and anal regions.<sup>1–3,5,7,9,13,14</sup> In 2 cases in which substantial resections were performed early, a detailed analysis of the resected tissues showed that the lumen was strictured or completely lost, intestinal layers showed fibrosis, calcified fibrotic areas, reduced lymphoid follicle size, and a thickened bowel wall suggestive of chronic intestinal inflammation.<sup>15</sup> Furthermore, tissue analysis of resected duodenal atretic areas showed ulcerations in the mucosa, loss of epithelial polarity, mucosal sloughing, apoptotic bodies, and loss of villi architecture, which were commonly reported features in patient histology.<sup>15</sup> Along with MIA, patients showed other intestinal defects, including meconium peritonitis, omphalocele, and bowel distension.<sup>1,7,15</sup>

Less than 20% of TTC7A patients presented with MIA alone.<sup>11</sup> In the general population, the development of MIA-CID is considered sporadic, where 10%-15% of MIA patients concomitantly present with CID.<sup>2,11,14</sup> The incidence of MIA accompanying CID is higher, approximately 50%, in TTC7A patients.<sup>11,14</sup> Although no TTC7A-deficient patients were reported to have isolated CID, the immune defect accompanied intestinal disease in more than 75% of cases, pointing toward a crucial role for TTC7A in maintaining intestinal and immune homeostasis.<sup>5,11,16</sup> Intestinal disease manifestations can present as early as in utero, whereas CID may be difficult to recognize at very early ages. The high incidence of CID in reported cases suggests that rigorous testing must be performed to exclude CID in TTC7Adeficiency confirmed patients. Lymphocytopenia in combination with disruption of the epithelial barrier results in increased risk for pathogenic proliferation and infection, and, consequently, many TTC7A-related fatalities have resulted from sepsis.<sup>3-6,11,14</sup> MIA-CID patients were reported to have infections caused by various forms of enteric microbes, including but not limited to: Escherichia coli, Enterococcus fecalis, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, and Klebsiella pneumoniae.<sup>2–7,9,11,14,15,48</sup> Several groups attributed barrier dysfunction as infections.<sup>2,7,11,15</sup> а possible cause for recurrent

Patients with CID were found to have a hypoplastic thymus, which is thought to disrupt T-cell maturation and result in lymphocytopenia.<sup>3,5,6</sup> To that end, Chen et al<sup>2</sup> observed that patients with milder T-cell lymphocytopenia had increased thymic function and integrity; furthermore,

they hypothesized that CD8<sup>+</sup> T-cell lymphocytopenia points to a defect in thymopoiesis, a chief function of the thymus in which thymocytes differentiate to mature T cells. Low blood immunoglobulin levels (ie, IgA, IgM, IgG) also were suggestive of reduced B cells or B-cell functioning.<sup>3,6,8,11,13,14</sup> In light of the presence of naive B cells, Woutsas et al<sup>8</sup> suggested that patients with hypomorphic mutations may have compromised B-cell development owing to increases in the relative levels of transitional B cells. Regulating PI4P levels via scaffolding of the PI4KIII $\alpha$  complex likely is compromised in patients with hypomorphic forms of TTC7A, which is thought to down-regulate activities in the PIP pathway, and in turn may affect B-cell-receptor assembly and organelle synthesis in activated B cells.<sup>8,49</sup> Ultimately, more research is needed to understand how TTC7A supports lymphocytic immunity and whether CID stems from errors in intrinsic lymphocytic functioning or extrinsic factors influencing lymphocytic progenitors, proliferation, and/or maturation.

It is unclear whether the disease pathogenesis results from abnormalities in embryonic development, the immune system, or the intestinal epithelium. To shed light on what could be driving the disease, 1 study showed that despite lacking lymphocytic immunity, mice with the flaky skin mutation (Ttc7 fsn/fsn) acquire epithelial defects, whereas transplantation of  $Ttc7^{fsn/fsn}$  hematopoietic cells alone did not confer disease phenotypes.<sup>50</sup> Moreover,  $Ttc7^{fsn/fsn}$  lymphocytes intensified epithelial hyperplasia and inflammation.<sup>50</sup> Taken together, these results imply that immune defects caused by TTC7A deficiency worsen epithelial disease, but may not be the primary cause.

#### VEOIBD With or Without CID

Approximately half of the reported TTC7A cases involve symptoms that align with clinical features associated with monogenic IBD or VEOIBD.<sup>1-3,6,8,11,13</sup> The designation of VEOIBD is given to children younger than age 6 years presenting with combinations of autoimmunity; primary immunodeficiency; lymphoid mucosal infiltration; ileal/ general colonic inflammation; histologic features in line with Crohn's disease, ulcerative colitis, or inflammatory bowel disease unclassified; growth delay; penetrating lesions; stricturing; perianal disease; and/or diarrhea.42,51 Rare variants in TTC7A have been uncovered in the most severe forms of VEOIBD.<sup>3,13,51</sup> Fewer than 10% of TTC7Adeficiency patients have VEOIBD alone and the majority have chronic intestinal inflammation accompanying CID and/or MIA.11 The severity and extent of gastrointestinal tract inflammation varies between the adult and pediatric IBD subset, in which approximately 43% of children have extensive ileocolonic inflammation compared with 3% in the adult group.<sup>40</sup> Furthermore, the younger the children at the age of diagnosis, the greater the proportion (one third) with inflammatory bowel disease unclassified phenotypes, highlighting the diverse phenotypic range of VEOIBD disease features.<sup>42</sup> TTC7A-deficiency patients with VEOIBD showed chronic intestinal inflammation, secretory diarrhea from birth, hematochezia, loss of intestinal architecture villi/microvilli, pseudostratified affecting epithelia,

intestinal epithelial cell sloughing, friable mucosa, ulcerations, apoptotic enterocolitis, lymphocytopenia, and hypogammaglobulinemia.<sup>3,13,51</sup> In an attempt to treat the intestinal disease, many TTC7A patients receive total parenteral nutrition;<sup>3,5,6,11,14</sup> however, avoiding enteral feeding alone does not treat the severe intestinal disease.

The histologic and endoscopic analyses of some patients were reported to be reminiscent of intestinal graft-versushost disease, a destructive pathologic condition associated with apoptotic crypts and resulting from allogenic HSCT.<sup>3,8</sup> In particular, disease features tied to a loss of epithelial barrier function, such as diarrhea, intestinal sloughing, and loss of intestinal architecture, were reported frequently and are present in both IBD and graft-versus-host disease.<sup>52,53</sup> Consequently, it is difficult to differentiate between the source of barrier dysfunction in IBD and graft-versus-host disease. Although IBD and graft-versus-host disease share similarities, several differences exist; for example, bloody and watery diarrhea tend to be associated with IBD and graft-versus-host disease, respectively, and this may serve to explain why many TTC7A-deficiency patients have been classified as having symptoms more in line with monogenic IBD or VEOIBD.<sup>52</sup> More patient cases and disease modeling will help to better define the intestinal pathogenicity driving TTC7A deficiency, which may be useful in the identification of effective therapies.

#### Increased Intestinal Epithelial Cell Apoptosis

Histologic analysis of patient biopsy samples, via H&E staining, shows that increases in intestinal epithelial cell apoptosis is a pathophysiologic feature shared among TTC7A-deficient patients.<sup>3,5,6,8</sup> Concomitant with MIA, CID, and IBD, intestinal epithelial cell apoptosis in crypts (ie, gland destruction) and villi are thought to contribute significantly to the enteropathy.<sup>3,8,14</sup> Apoptotic enterocolitis, used to describe several TTC7A patients, is a feature pathologically similar to necrotizing enterocolitis as well as graft-versus-host disease, both of which are life-threatening conditions resulting in epithelium deterioration.<sup>53,54</sup> It is tempting to surmise that the increase in apoptosis, in contrast to necrosis, points to TTC7A having a role in promoting cell viability or antagonizing apoptotic activation pathways. Tightly regulated apoptosis is fundamental to epithelial cell renewal and epithelium homeostasis, however, increased intestinal epithelial cell apoptosis can disrupt epithelial barriers, allowing translocation of bacteria and antigens into the lamina propria, leading to inflammation and tissue destruction.55,56

## **Extraintestinal Manifestations**

Extending the phenotypic repertoire associated with TTC7A deficiency, several extraintestinal manifestations related to integumentary hyperplasia (ie, affecting the dermis) and poor hepatic function have been reported.<sup>1–11,13,14,16</sup> Although many systems seem to be affected in TTC7A deficiency, immune and intestinal tract defects are the most formidable, hinting that compensatory mechanisms may exist in other specialized cells, reducing

the apparent severity of extraintestinal phenotypes. However, data functionally validating extraintestinal disease features are lacking and it remains to be confirmed whether TTC7A deficiency is the single underlying cause for the diverse range of symptoms reported in this cohort. Neves et al<sup>13</sup> reported a TTC7A patient presenting with VEOIBD, MIA-CID, and trichohepatoenteric syndrome, a rare genetic condition typically caused by mutations in TTC37. Trichohepatoenteric syndrome is associated with multiple abnormalities of the hair and liver, as well as chronic diarrhea, thyroiditis, hypogammaglobulinemia, ichthyosis (scaly skin), café au lait spots, facial dysmorphia, and enamel erosion of teeth.<sup>13</sup> The complexity associated with TTC7A pathophysiology is exemplified further by a large consanguineous family of 13 affected individuals, possessing the same genotype, and presenting with enterocolitis-lymphopeniaalopecia syndrome.<sup>6</sup> Although the severity of intestinal phenotypes varied within this family, morbidity typically decreased with age. Patients older than 4 years of age no longer required total parenteral nutrition, however, they became affected by dermatologic features such as epidermal hyperplasia and ichthyosis.<sup>6</sup> Extraintestinal disease phenotypes seem to be more abundant in older surviving patients, suggestive of an inverse relationship between intestinal disease severity and extraintestinal manifestations.

## Therapies and Outcomes

There is no standard of care for TTC7A deficiency and traditional IBD therapies do little to treat the intestinal disease (ie, MIA and VEOIBD).<sup>10,11</sup> To date, there is no known treatment for MIA and surgical resections do not prevent the formation of new atresias.<sup>57</sup> Chronic diarrhea and intestinal epithelial cell apoptosis typically are refractory to immunosuppressives, steroids, and biologics.<sup>3,58</sup> Recently, Fayard et al<sup>15</sup> reported 2 unrelated patients with mild MIA, severe and progressive IBD, and severe CID who were treated successfully with early total enterectomy, parenteral nutrition, and prophylaxis immunoglobulin (Privigen, Ottawa, Ontario) and antibiotic (Bactrim, Philadelphia, PA) treatments. Patients with MIA-IBD-CID typically have poor outcomes (mean survival, 24 mo), yet at publication, these patients were ages 48 and 18 months and showing positive clinical responses. Alternatively, a smallbowel-liver transplant restored intestinal function, normal feeding, and improved CD3<sup>+</sup> and CD19<sup>+</sup> lymphocytes in a 16-month-old patient with MIA-CID; unfortunately, no genetic background was provided for this patient.<sup>59</sup> According to reported cases, no TTC7A-deficiency patient has received an intestinal transplant, however, apart from few donors, this intervention is associated with high mortality rates.<sup>60</sup>

## HSCT Does Not Treat the Intestinal Disease in TTC7A Deficiency

Identifying the cause of immune dysfunction can inform clinical action. For example, HSCT can be completely restorative for VEOIBD patients with interleukin 10 defects, whereas the benefit of the same intervention seems to be unclear for TTC7A patients.<sup>61</sup> Beyond the benefit of immune reconstitution, it was shown that HSCT potentially could benefit recipient intestinal epithelial cells through donor/ recipient cell fusion.<sup>62</sup> In a TTC7A patient follow-up evaluation after HSCT, it was reported that 4 patients, at various ages (2 patients younger than 1 year and 2 patients younger than 2 years), likely had immune reconstitution after transplant.<sup>10</sup> However, 3 of these patients required subsequent resection surgeries for recurrent stricturing, 2 required immunosuppression therapy, all patients required intravenous nutrition, and all patients had unresolved intestinal inflammation. Six more TTC7A patients with HSCT were reported, but aberrant intestinal phenotypes persisted beyond the transplant and 5 of 6 patients died.<sup>2,4,5</sup> HSCT is thought to correct immune defects and may increase the survival of patients with immunodeficiency, however, it does not seem to improve phenotypes related to intestinal epithelial defects.<sup>10,11</sup> To further complicate the risk-benefit analysis of HSCT, the deaths of 2 TTC7A-deficiency patients were attributed to HSCT, while graft-versus-host disease and sepsis are potential complications that can exacerbate existing intestinal phenotypes.<sup>6,11</sup> Aside from the possibility of small-bowel transplantation, no other therapy exists to target and resolve TTC7A-related epithelial defects.

#### Patient Outcomes

TTC7A patients are a phenotypically diverse group, and although some symptoms are less severe than others, young patients tend to have refractory intestinal disease features with high mortality rates.<sup>1–11,13,14,16</sup> Lien et al<sup>11</sup> analyzed 49 previously reported TTC7A patients to identify genotype and phenotype associations. In the cohort, 41% of TTC7A patients survived and approximately half of this group had IBD. Prominent phenotypes among the 49 patients included the following: MIA-CID (33%), IBD-CID (29%), MIA (16%), MIA-CID-IBD (16%), and IBD (6%). Sepsis, bowel obstruction, viral pneumonia, and HSCT were among the top causes of mortality in these patients. In the (aforementioned) consanguineous family of 13, 5 patients died of the following causes: HSCT (2 patients, <1 y), enteropathy (1 patient, <1 y), sepsis (1 patient, 4 y), and gastric adenocarcinoma (1 patient, 14 y).<sup>6</sup> From this large family, the range of outcomes affecting the intestinal and immunologic milieu can be appreciated, showing that TTC7A disease severity can depend on, but is not limited to, genetics.

## Disease Modeling to Study TTC7A Deficiency

Finding a suitable animal model to study TTC7A deficiency has not been straightforward. For example, a spontaneous mutation in the *Ttc7* gene resulted in a prominent flaky skin (ie, *fsn*) phenotype in mice, which resembled human psoriasis.<sup>20</sup> The *Ttc7*<sup>fsn</sup> mice also presented with multisystem defects, including anemia, testicular degeneration, CD4/CD8 imbalance, and apoptotic cecal intestinal epithelial cells.<sup>20</sup> Sequencing of *Ttc7* showed an insertion of an additional exon into a TPR domain, resulting in decreased transcripts and reduced function of Ttc7 in *fsn*  mice.<sup>20</sup> Furthermore, Takabayashi et al<sup>63</sup> compared *Ttc7<sup>fsn</sup>* mutants and found that the mutants with large deletions spanning exons 1-14, specifically affecting exons 9 and 10, had worse disease phenotypes and mortality. Although psoriasis is a condition driven by autoimmunity, the lack of single-nucleotide polymorphisms in TTC7A associated with psoriasis in human beings, and the lack of intestinal atresias in mice, suggests varying functional specificities between the human and mouse orthologs.<sup>64,65</sup> Although the dermatologic abnormalities in  $Ttc7^{fsn}$  mice are reminiscent of the ichthyosis and psoriasis in some TTC7A-deficiency patients,<sup>6,66</sup> the mice do not seem to recapitulate the intestinal disease in patients. Establishing mutant mice with a wide variety of knock-in Ttc7 mutations, developed via clustered regularly interspaced short palindromic repeats (CRISPR)/ CRISPR-associated protein 9 (CAS9) editing for example, will allow for a thorough evaluation of mice as a disease model to study the intestinal defects caused by TTC7A deficiency.

In the past 20 years, zebrafish (Danio rerio) have been established as relevant models to study human disease.<sup>67</sup> Zebrafish serve as an economic model to bridge the gap between cellular and classic mammalian models. The intestinal tracts of zebrafish and human beings have homologous structures, functions, tissues, and cell types, however, the transparency of zebrafish during the larval stage makes them an advantageous model for intestinal research.<sup>68</sup> Zebrafish have been established as an effective model for studying intestinal development and IBD.<sup>69,70</sup> Furthermore, the epithelial changes seen in zebrafish after chemically induced inflammation are reminiscent of the changes observed in mouse models.<sup>69,70</sup> Likewise, the use of antiinflammatories, steroids, and antibiotics also were successful in abrogating inflammatory features.<sup>69,70</sup> Similar to mice that incur gastrointestinal defects via Pi4kIII $\alpha$  induced knockout, zebrafish lacking de novo synthesis of phosphatidyl inositol (ie, *cdipt*<sup>hi559</sup> mutants), a precursor for PI4P, had aberrant intestinal histopathologic features, including villous atrophy, loss of intestinal architecture, intestinal epithelial cell apoptosis, and a narrow intestinal lumen.<sup>11</sup> Ttc7a mutant zebrafish were described in a thesis entitled Congenital Diseases of the Intestine by Halim in 2016, in which the author created mutant zebrafish with indels in exon 3 of *ttc7a* (no genotyping provided).<sup>72</sup> Motility assays using fluorescent microspheres showed that 46% of homozygous mutants, compared with 37% wild-type/ heterozygous fish, failed to excrete the fluorescent microspheres, showing increased transit times (and decreased gut motility) in homozygous ttc7a fish.<sup>72</sup> The ttc7a mutant zebrafish further implicate TTC7A in driving intestinal defects, and provide a precedence for using zebrafish as a vertebrate model to study TTC7A deficiency.<sup>72</sup>

As an ex vivo model, enteroids serve to bridge the gap between cell-based and in vivo models. The seminal work of the Clevers group established protocols for culturing primary tissue-derived intestinal organoids from mouse intestinal tissue samples.<sup>73</sup> Because organoids can be cultured directly from patient stem cells, they provide a tremendous platform for the development of personalized medicine.<sup>74</sup> Limited data have been published on TTC7A patient-

derived organoids, partly because of culturing difficulties. To date, organoids derived from patients with hypomorphic TTC7A variants (in contrast to truncated variants) seem to be the most viable in ex vivo culture.<sup>5,6</sup> TTC7A organoids have been used in 2 studies from Bigorgne et al<sup>5</sup> and Lemoine et al,<sup>6</sup> and have confirmed that the epithelial defect exists in isolation from the microbiome and aberrant immune responses. Morphology and proliferation of intestinal organoids have been assessed via immunofluorescence staining with various markers. Polarity markers have included filamentous actin,  $\alpha 6$  integrin, zonula occulens tight junction protein, and E-cadherin, all showing mislocalization in TTC7A organoids.<sup>5,6</sup> Rescue of the polarity defect in TTC7A patient-derived organoids with a small molecule ROCK inhibitor provides proof-of-principle that epithelial phenotypes specific to TTC7A can be abrogated therapeutically.<sup>5,</sup>

## **Conclusions and Future Directions**

Rare autosomal-recessive variants in TTC7A have been uncovered in the most severe and fatal forms of pediatric intestinal disease. Advancements in genetic diagnosis have facilitated the identification of more than 50 patients with TTC7A-derived monogenic disease, presenting with diverse phenotypes, including but not limited to, intestinal and immune aberrations. Although the specific targets and interactions of TTC7A remain unknown, existing biochemical and organoid research provides evidence for TTC7A in maintaining intestinal epithelial cell integrity. Zebrafish represent an emerging animal model with the potential to recapitulate intestinal disease features caused by TTC7A deficiency. Currently, no treatment exists that can maintain clinical remission in TTC7A patients. Given that rare monogenic diseases represent good candidates for the development of personalized medicine, zebrafish and patient-derived organoids are possible platforms for drug discovery.

Many important questions exist for future researchers and clinicians to address: how do specific mutations alter TTC7A's secondary structure and scaffolding ability? What animal models can appropriately recapitulate the spectrum of intestinal disease features? Given that patients had various combinations of MIA and/or IBD, with or without CID, what is the threshold for the development of atresias, inflammation, and immunodeficiency? How does TTC7A deficiency result in T-cell and B-cell lymphocytopenia, is it a lymphocyte production, destruction, and/or maturation defect? What is the scope of TTC7A-deficiency etiology and how do other factors (ie, genetic milieu, environment, lifestyle, microbiome, immune system) influence patient outcomes? What phenotypes and/or key cellular players might be targets in TTC7A-deficiency drug discovery? Given the rarity of diseases such as TTC7A deficiency, we are provided with clear challenges in that we know relatively little regarding the breadth and depth of TTC7A function and that patient cases to date are too few to make significant genotype/phenotype correlations. Nevertheless, as the global prevalence of IBD is increasing, decoding the pathways and targets of TTC7A may provide more insight into the subtle inner workings of intestinal health and its interplay with the immune system. Understanding TTC7A's pathobiology may lead to the development of effective therapies capable of improving intestinal health and that have the potential to benefit patients in the broader IBD pool.

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#### Received October 25, 2018. Accepted December 6, 2018.

#### Correspondence

Address correspondence to: Aleixo M. Muise, MD, PhD, FRCPC, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8. e-mail: aleixo.muise@sickkids.ca; fax: (416) 813-6531.

#### The authors disclose no conflicts.

Conflicts of interest

#### Funding

Funded by the Canadian Institute for Health Research and the Leona M. and Harry B. Helmsley Charitable Trust (A.M.M.).