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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 IIIα; 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.**

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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.[1] 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.[2,3] Recently, several groups, including our own, demonstrated that mutations in *IL10RA/B* genes[4] cause a severe form of VEOIBD, with symptoms consistently developing in infancy.[5] Subsequently, causative variants in *IL10*,[6] *XIAP*,[7] *ADAM*17,[8] and *NCF4*,[9] and association variants in the nicotinamide adenine dinucleotide phosphate oxidase genes *NCF2/RAC2*[10] were identified in VEOIBD patients, suggesting that severe infantile colitis frequently starting immediately after birth might represent a group of heterogeneous monogenetic 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.[11,12] 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.[13]

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-phycoerythrin 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