Immune deficiency–related enteropathy-lymphocytopenia-alopecia syndrome results from tetratricopeptide repeat domain 7A deficiency

Roxane Lemoine, PhD,[a,b,c] Jana Pachlopnik-Schmid, MD, PhD,[a,b,c‡] Henner F. Farin, PhD,[d*] Amélie Bigorgne, PhD,[a,b,c*] Marianne Debré, MD,[c] Fernando Sepulveda, PhD,[a,b,c] Sébastien Héritier, MD,[c] Julie Lemale, MD,[e] Cécile Talbotec, MD,[f] Frédéric Rieux-Laucat, PhD,[a,b,c] Frank Ruemmele, MD,[f] Alain Morali, MD,[g] Pascal Cathebras, MD,[h] Patrick Nitschke, PhD,[b] Christine Bole-Feysot, PhD,[b] Stéphane Blanche, MD,[a,b] Nicole Brousse, MD,[b,i] Capucine Picard, MD, PhD,[b,c,j] Hans Clevers, MD, PhD,[d] Alain Fischer, MD, PhD,[a,b,c,k] and Geneviève de Saint Basile, MD, PhD[a,b,c,j]

Paris and Saint Etienne, France, and Utrecht, The Netherlands

Background: Inflammatory bowel disease (IBD) is one of the most common chronic gastrointestinal diseases, but the underlying molecular mechanisms remain largely unknown. Studies of monogenic diseases can provide insight into the pathogenesis of IBD.

Objective: We thought to determine the underlying molecular causes of IBD occurring in 2 unrelated families in association with an immune deficiency.

Methods: We performed genetic linkage analysis and candidate gene sequencing on 13 patients from a large consanguineous family affected by early-onset IBD, progressive immune deficiency, and, in some cases, autoimmunity and alopecia, a condition we named enteropathy-lymphocytopenia-alopecia. The candidate gene was also sequenced in an unrelated patient with a similar phenotype. We performed histologic analysis of patients' intestinal biopsy specimens and carried out functional assays on PBMCs. Gut organoids derived from a patient's biopsy specimen were analyzed.

Results: We identified biallelic missense mutations in tetratricopeptide repeat domain 7A *(TTC7A)* in all patients from both families. The resulting TTC7A depletion modified the proliferation, adhesion, and migratory capacities of lymphocytes through inappropriate activation of the RhoA signaling pathway. Normal function was restored by wild-type *TTC7A* expression or addition of a RhoA kinase inhibitor. The growth and polarity of gut epithelial organoids were also found to be dependent on the RhoA signaling pathway.

Conclusions: We show that TTC7A regulates the actin cytoskeleton dynamics in lymphocytes through the RhoA signaling pathway and is required in both lymphocytes and epithelial cells for maintaining equilibrium between cell proliferation, migration, polarization, and cell death. Our study highlights variability in the phenotypic expression resulting from TTC7A deficiency and outlines that impairment of both epithelial cells and lymphocytes cooperatively causes IBD. (J Allergy Clin Immunol 2014;134:1354-64.)

Key words: *Inflammatory bowel disease, immune deficiency, tetratricopeptide repeat domain 7A deficiency, lymphocytes, cytoskeleton, gut organoid, RhoA kinase, cell homeostasis*

Inflammatory bowel disease (IBD) is one of the most common chronic gastrointestinal diseases in the developed world.[1,2] It comprises a heterogeneous group of disorders that differ in terms of the gastrointestinal sites involved and the characteristics of the inflammation. Ulcerative colitis and Crohn disease (CD) are the predominant subtypes of IBD.[3] Whereas ulcerative colitis is confined to the colon, CD can occur anywhere along the gastrointestinal tract. Experimental studies and genetic evidence suggest that chronic intestinal inflammation can be triggered by various environmental factors in genetically susceptible subjects.[4-6] Maintaining a normal balance between competence to respond to intestinal pathogens and the suppression of inflammatory responses to commensal microbes depend on (1) the integrity of the mucosal barriers,[6,7] (2) the activity of proinflammatory signaling pathways,[8] and (3) the regulation of innate and adaptive immune responses in the intestine and draining lymphoid organs.[9] Defects in these components have been implicated in patients

From [a]INSERM, Unité U1163, Laboratoire Homéostasie normale et pathologique du système immunitaire, Hôpital Necker Enfants-Malades, Paris; [b]Université Paris Descartes-Sorbonne Paris Cité, Institut Imagine, Paris; [c]Unité d'Immunologie et Hématologie Pédiatrique, Assistance Publique-Hôpitaux de Paris, Hôpital Necker Enfants-Malades, Paris; [d]the Hubrecht Institute for Developmental Biology and Stem Cell Research and University Medical Centre Utrecht; [e]Unité de Nutrition et Gastroentérologie Pédiatriques, Hôpital Trousseau, Paris; [f]Service de gastroentérologie, hépatologie et nutrition pédiatriques, Hôpital Necker-Enfants Malades, Paris; [g]Unité d'Hépato-Gastro-Entérologie et Nutrition Pédiatriques, Hôpital d'Enfants, CHU Nancy Brabois–F 54500 Vandoeuvre les Nancy et Unité INSERM U954; [h]Service de Médecine Interne, CHU de Saint Etienne, Saint Etienne; [i]Laboratoire d'Anatomie Pathologique, Assistance Publique-Hôpitaux de Paris, Hôpital Necker, Paris; [j]Centre d'Etudes des Déficits Immunitaires, Assistance Publique-Hôpitaux de Paris; and [k]College de France, Paris.

*These authors contributed equally to this work.

‡Dr Pachlopnik-Schmid is now with the Immunology Department, University Children's Hospital Zurich, Zurich, Switzerland.

Supported by INSERM, Agence Nationale de la Recherche-12-BSV1-0020-01, and an advanced grant from the European Research Council (ERC; PIDImmun [ref 249816]), the ARC Foundation (R2011 confocal equipment), and the Imagine Foundation. J.P.-S. was supported by the Swiss National Science Foundation (SNSF), and F.S. was supported by the ARC Foundation and Becas Chile.

Disclosure of potential conflict of interest: R. Lemoine and A. Fischer have received research support from the European Research Council (ERC PIDImmun 249816). J. Pachlopnik-Schmid has received research support from the Swiss National Science Foundation, has been employed by INSERM, and is currently employed by University Children's Hospital in Zurich, Switzerland. A. Bigorgne and G. de Saint Basile have received research support from Agence Nationale de la Recherche (12-BSV1-0020-01). The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication February 19, 2014; revised June 4, 2014; accepted for publication July 12, 2014.

Available online August 28, 2014.

Corresponding author: Geneviève de Saint Basile, MD, PhD, INSERM U768, Hôpital Necker Enfants-Malades, 149 rue de Sèvres, Paris, F-75015, France. E-mail: genevieve.de-saint-basile@inserm.fr.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2014.07.019

Abbreviations used
CD: Crohn disease
CMV: Cytomegalovirus
ELA: Enteropathy-lymphocytopenia-alopecia
HSCT: Hematopoietic stem cell transplantation
IBD: Inflammatory bowel disease
NK: Natural killer
p-ERM: Phospho–ezrin-radixin-moesin
p-MLC: Phospho-myosin light chain 2
ROCK: RhoA kinase
SNP: Single nucleotide polymorphism
TPR: Tetratricopeptide repeat
TTC7A: Tetratricopeptide repeat domain 7A

with IBD, although our fundamental knowledge of the underlying disease mechanism remains patchy.

Over the last decade, genetic studies have emphasized the role of host susceptibility in the onset of IBD. About 163 risk loci containing several genes have been identified,[10] most of which encode proteins involved in immunity, host defense against microbes, and/or gut epithelium renewal.[11-14] However, when considered individually, these loci only procure a minor relative risk.[14] Nucleotide-binding oligomerization domain 2 *(NOD2)*, a pattern recognition receptor involved in proinflammatory and anti-inflammatory cytokine production, as well as in autophagy induction, is the best-established susceptibility gene for CD.[15]

Highly penetrant monogenic causes of IBD also exist but are rare. It has been shown that homozygous null alleles of IL-10, IL-10 receptor α, and IL-10 receptor β, which are required to prevent an excessive immune response, cause very early-onset IBD.[16,17] Furthermore, a proportion of patients with X-linked inhibitor of apoptosis deficiency had a severe CD-like disease.[18] X-linked inhibitor of apoptosis is an apoptosis inhibitor involved in NOD2's downstream activation pathway.[19] Genetic mutations in components of the nicotinamide adenine dinucleotide phosphate oxidase complex that impair the respiratory burst in phagocytic leucocytes and cause chronic granulomatous disease are also associated in a high proportion of patients with CD-like IBD.[20] Recently, recessive mutations in the tetratricopeptide repeat domain 7A *(TTC7A)* gene were reported in patients with very early-onset IBD.[21] Interestingly enough, null mutations of TTC7A deficiency are the cause of combined immune deficiency with multiple intestinal atresia.[22-24]

Here we show that an early-onset IBD associated with progressive immune deficiency and eventually alopecia, as observed in 14 patients from 2 unrelated families, results from biallelic missense mutations in *TTC7A*. TTC7A deficiency causes inappropriate activation of RhoA-dependent effectors regulating cytoskeletal dynamics. This activation alters cell polarization, adhesion, and proliferation in lymphocytes and gut epithelial cells. To our knowledge, this condition is the first in which both impairments of lymphocyte functions and epithelial cells contribute to an IBD, although determination of their respective roles will require additional work.

METHODS

Details of the methods used in this study are provided in the Methods section in this article's Online Repository at www.jacionline.org. Clinical information and blood samples were collected from the patients, their relatives, and control subjects, all of whom had given their prior informed consent to participate in the study. Genetic studies and data collection procedures were approved by the local investigational review board and the French Advisory Committee on Data Processing in Medical Research.

RESULTS

Manifestations of the enteropathy-lymphocytopenia-alopecia syndrome

Thirteen members of a large consanguineous kindred displayed enteropathy associated with T-cell, B-cell, and natural killer (NK) cell combined immunodeficiency. The family members' pedigree and clinical features are shown in Fig 1, Table I, and Table E1 in this article's Online Repository at www.jacionline.org. All patients had IBD within the first days to months of life and displayed recurrent severe diarrhea that was sometimes associated with rectal bleeding and weight loss. Enteral or total parenteral nutrition were repeatedly required in 9 patients until age 5 years (Fig 1, *C*, and Table I). Enteropathy was fatal in 1 patient (patient E3). Of note, the severity of gastrointestinal manifestations gradually decreased with age, and none required total parenteral nutrition beyond 4 years of age. Five patients had alopecia from the age of 2 to 4 years (Fig 1, *C*), and 4 had onychopathy. Patients had autoimmune manifestations from the age of 10 years (Fig 1, *C*), including autoimmune hepatitis in patient B4, autoimmune hemolytic anemia in patient A3, psoriasis and type I diabetes in patient B4, and autoimmune thyroiditis in patient C1. Two patients (patients N3 and F5) underwent hematopoietic stem cell transplantation (HSCT). Patient N3 died soon after, whereas patient F5 died 9 months later from infection. It is noteworthy that the gastrointestinal disease in patient F5 was not resolved by HSCT, despite full donor chimerism and the absence of skin or liver graft-versus-host manifestations. This observation suggests the presence of an intrinsic gut disease.

Endoscopy showed severe inflammation with an erythematous stomach and multiple areas of ulcerative lesions in the sigmoid, as exemplified in Fig 2, *A* (patient O3). Immunohistopathologic analysis of the digestive tract revealed major lesions in the antrum and colon in all patients (Fig 2, *B*, *a* and *b* and *e* and *f*). In contrast, the small intestine was relatively unaffected (Fig 2, *B*, *c* and *d*). This finding might be related to the redundant function of the related TTC7B molecule that is highly expressed in this tissue.[25] The fundus and antrum were characterized by changes in the surface and foveolar epithelium that were exactly the same in all patients analyzed at different ages (Fig 2, *B*, *a* and *b*). The disorganized architecture of the epithelium consisted of tufting, loss of apical mucin, and abnormal pseudostratified cell organization with glandular architectural distortion and epithelial apoptosis (Fig 2, *B*, *a* and *b*). The lamina propria contained an inflammatory infiltrate with mononuclear cells and a high eosinophil count. Antrum sections were *Helicobacter pylori* negative. Colon lesions were severe and widespread. The epithelium was dedifferentiated, and little mucus-secreting tissue remained. A combination of glandular necrosis, cell apoptosis, and crypt abscesses in multiple sites was also noticed (Fig 2, *B*, *e* and *f*, and see Fig E1, *A*, in this article's Online Repository at www.jacionline.org). The lamina propria was infiltrated by polymorphous inflammatory cells, including mononuclear cells (CD4, CD8 T cells, B cells, and macrophages) and