

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

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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;■■■■:■■■■-■■■■.)

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

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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

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

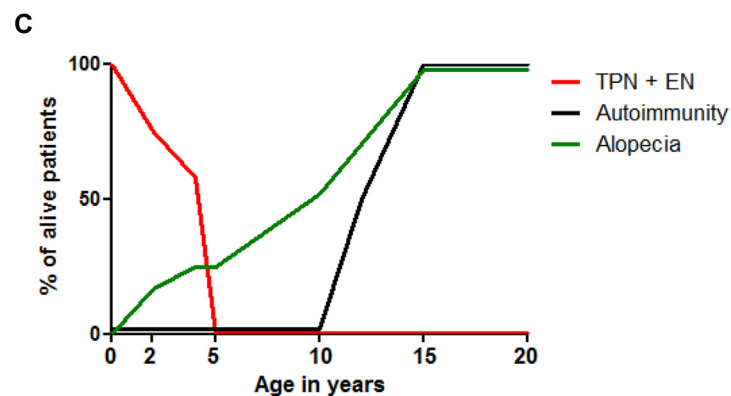
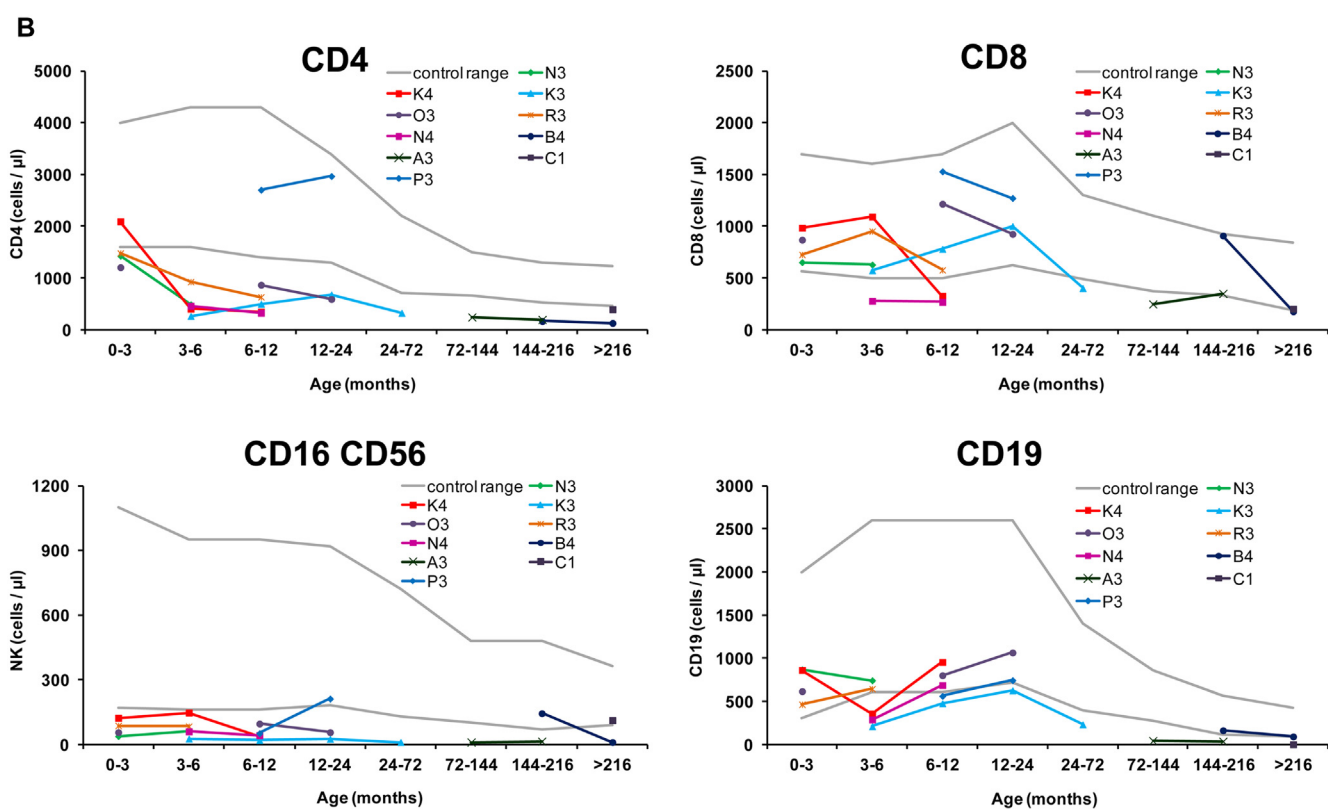
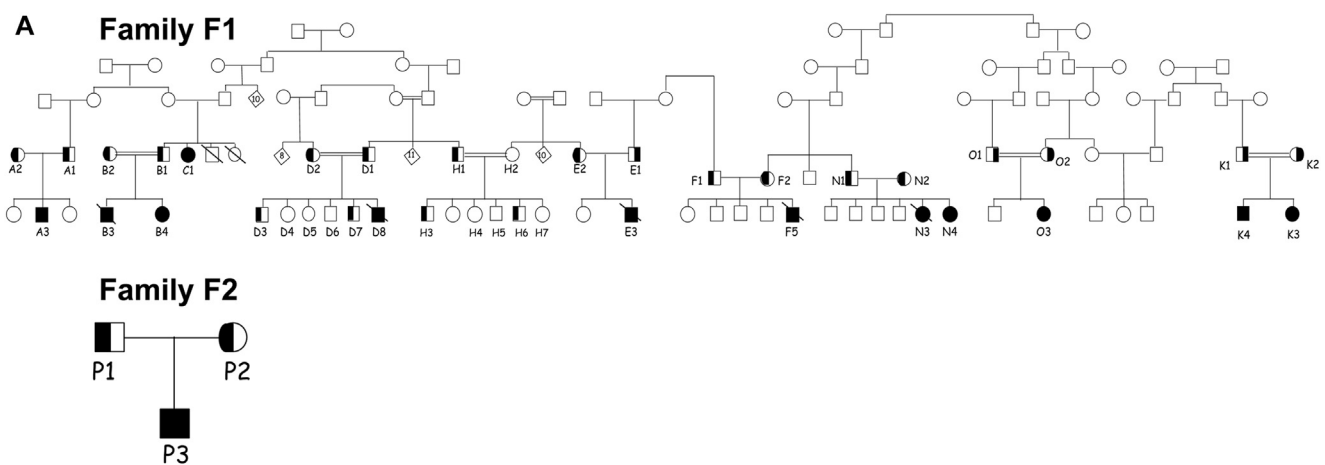


TABLE I. Summary of patients' clinical characteristics

Family ID	F1	F1	F1	F1	F1	F1	F1	F1	F1	F1	F1	F1	F1	F2
Patient ID	R3	N3	F5	E3	K4	N4	O3	K3	D8	B3	A3	B4	C1	P3
Sex	F	F	M	M	M	M	F	F	M	M	M	F	F	M
Age at last follow-up	5 mo	6 mo	6 mo	8 mo	10 mo	10 mo	2 y	4 y	4 y	14 y	14 y	28 y	50 y	18 mo
IBD	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Enteral nutrition	—	—	—	—	+	—	—	+	—	—	—	—	—	+
Total parenteral nutrition	+	+	+	+	—	—	+	—	+	+	—	—	—	—
Recurrent upper (U) and lower (+) respiratory tract infections	U	NE	+	—	NE	—	—	NE	NE	+	+	+	+	+
CMV infection	+	—	+	+	+	—	+	+	—	—	—	—	—	—
Alopecia	—	—	—	—	—	—	—	+	—	+	+	+	+	+/-
Onychopathy	—	—	—	—	—	—	—	+	—	+	—	+	+	—
Hepatomegaly	+	—	+	—	—	—	+	—	+	+	—	—	—	+
Autoimmunity	—	—	—	—	—	—	—	—	—	AH	AIHA	IDDM, psoriasis	Thyroiditis	—
Outcome	Alive	Died soon after HSCT	Died 9 mo after HSCT	Died (enteropathy)	Alive	Alive	Alive	Alive	Died (septic shock)	Died (gastric adenocarcinoma)	Alive	Alive	Alive	Alive

AH, Autoimmune hepatitis; AIHA, autoimmune hemolytic anemia; F, female; IDDM, insulin-dependent diabetes mellitus; M, male; NE, not evaluable (because of pre-emptive IgG therapy).

polymorphonuclear cells, notably eosinophils (Fig 2, B, e and f, and see Fig E1, B).

The patients showed increased susceptibility to infections. From the first year of life onward, 6 of the 8 patients who were not receiving pre-emptive IgG replacement therapy displayed lower and/or upper respiratory tract infections, which eventually resulted in bronchiectasis in patient C1. IBD was combined with evidence of protracted cytomegalovirus (CMV) infection on the basis of PCR analyses of blood and gut samples in 6 of 13 cases. Diarrhea was not improved by anti-CMV treatment.

Immunologic studies (Fig 1, B, and see Table E1) revealed normal polymorphonuclear cell and monocyte counts and T-cell, B-cell, and NK cell lymphocytopenia (Fig 1, B). T-cell receptor α/β and T-cell receptor γ/δ cells were equally affected (data not shown). CD4 lymphocytopenia, notably naive T-cell lymphocytopenia, predominated (Fig 1, B, and see Table E1). The CD8 T-cell counts were close to those of age-matched control subjects. However, in older patients naive CD8 T-cell counts decreased, whereas effector memory phenotype CD8 T cells were overrepresented (Fig 1, B, and see Table E1). Counts of regulatory T cells, as identified based on forkhead box protein 3-positive CD25^{hi}CD4⁺ coexpression, were low (see Table E1), although with a normal suppressive function (data not shown). NK cell counts were also low in most patients (Fig 1, B, and see Table E1). The patients' B-cell counts were close to normal in early life but then decreased. Memory (CD27⁺) B-cell counts were always very low (Fig 1, B, and see Table E1). Hypogammaglobulinemia (IgM, IgG, and IgA) was noted, requiring immunoglobulin replacement therapy (see Table E1). Antibody responses could not be evaluated. In most patients

T cells exhibited normal proliferation in response to mitogens but low or no proliferation in response to antigens (including to CMV in chronically infected patients, see Table E1). It is noteworthy that a proportion of circulating T cells expressed HLA-DR independent of CMV infection status, suggesting *in vivo* T-cell activation (data not shown). Patient P3 from the second family (F2) exhibited a similar, although slightly milder, phenotype. He had a history of neonatal bloody diarrhea that became protracted and recurrent anal abscess. He received partial parenteral nutrition for 10 months, which was then replaced by enteral nutrition. At the last follow-up (18 months old), weight and height development were within the mean for age. Sparse hairs, including the absence of eyebrows, were noticed. Recurrent respiratory tract infections were found associated with hypogammaglobulinemia (Table I and see Table E1). T-lymphocyte, B-lymphocyte, and NK lymphocyte counts, as well as T-lymphocyte proliferation in response to mitogens and antigen, were normal (Fig 1, B, and see Table E1). Immunohisto-pathologic analysis of the digestive tract showed inflammatory infiltrate of the lamina propria with a high eosinophil count and a combination of glandular necrosis, cell apoptosis, and crypt abscesses at multiple sites (data not shown).

Identification of mutations in TTC7A

With a view to identifying the gene underlying this enteropathy-lymphocytopenia-alpecia (ELA) syndrome and assuming an autosomal recessive inheritance, we performed genome-wide homozygosity mapping with a single nucleotide polymorphism (SNP) array. This mapping revealed a common

FIG 1. Pedigree and immunologic features of patients with the ELA phenotype. **A**, Pedigree of the 2 families with ELA. *Solid boxes* and *circles* represent affected male and female subjects, respectively. *Half-solid symbols* indicate a heterozygous carrier. **B**, Changes in lymphocyte cell counts over time in TTC7A-deficient patients: CD4 T-cell, CD8 T-cell, NK cell (CD16CD56), and B-cell (CD19) populations. *Gray lines* delineate the normative upper and lower limits of the cell counts as a function of age. **C**, Evolution of clinical manifestations as a function of patient age: total parenteral nutrition (TPN) and enteral nutrition (EN [in red]), autoimmunity (in black), and alopecia (in green).

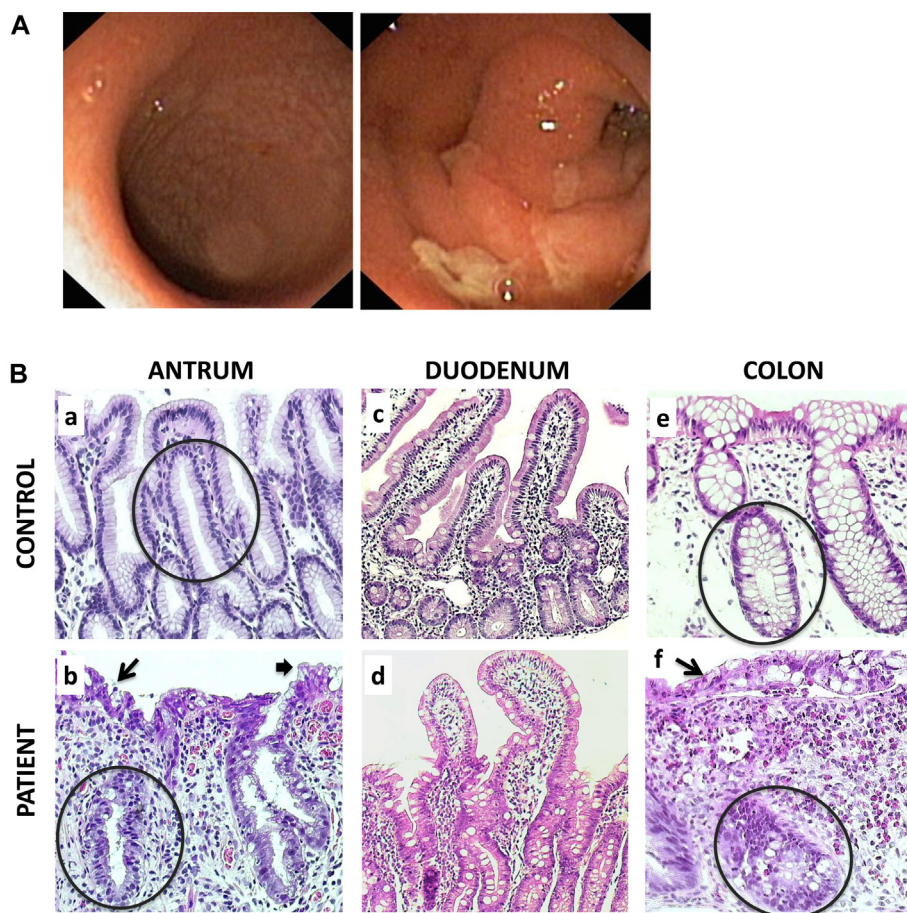


FIG 2. Endoscopic and immunohistologic analysis of the gut in patients with ELA. **A**, Endoscopic characteristics of the gastrointestinal tract from a patient with ELA (patient O3) showing erythematous lesions in the antrum (*left*) and ulcerative lesions in the sigmoid (*right*). **B**, Hematoxylin and eosin staining of the gastric antrum, duodenum, and colon in patients with ELA and control subjects. Arrows indicate the degenerative pseudostratified cell surface and gland epithelium. The *thick arrow* in Fig 2, B, indicates a tufting architecture. Circles highlight gland structures. Similar observations were made for 5 patients from the 2 families.

4-Mb region on chromosome 2p21-16.3 containing 23 annotated genes (see Fig E2, A, in this article's Online Repository at www.jacionline.org). Of the region's 23 genes, *TTC7A* appeared to be the most plausible candidate because (1) *TTC7A* is known to be expressed in lymphoid tissues and (2) *TTC7A* deficiency induces forestomach papillomas in the mouse. Sequencing of the *TTC7A* gene's 20 coding exons revealed a homozygous missense mutation in exon 2 in all 13 patients from family F1. The mutation prompts a 211G>A nucleotide change and an E71K amino acid substitution (see Fig E2, A). The latter glutamate acid residue is highly conserved in all species (data not shown) and predicted to be "probably damaging" (score = 0.997) by PolyPhen2, "deleterious" by SIFT (score = 0.00), and "disease causing" ($P = 1.0$) by MutationTaster. Exome sequencing in one of the patients also identified this substitution and showed that *TTC7A* was the only mutated gene within the defined genetic linkage region on chromosome 2p21-16.3. All tested parents were heterozygous for this mutation. When identified, the 211G>A mutation was not found in control subjects (486 chromosomes analyzed) and in any of the public databases (including the dbSNP129 and 1000 Genomes data sets). Heterozygous individuals were asymptomatic. Very

recently, this mutation was reported in association with a heterozygous nonsense mutation in *TTC7A* in a patient with a severe form of early-onset IBD associated with immune deficiency.²¹

In the patient from the second family, a heterozygous single nucleotide deletion (911delT) in exon 7 was identified. It was inherited from his father and predicted to lead to nonsense-mediated decay through a frameshift mutation (p.L304fsX59; see Fig E2). Patient P3 also carried a second heterozygous missense mutation in exon 12 inherited from his mother (1433T>C; see Fig E2, A). The latter mutation, p.L478P, is predicted to be "deleterious" by using SIFT, "possibly damaging" (score = 0.811) by PolyPhen2, and "disease causing" ($P = .784$) by MutationTaster tools.

Defective expression of the *TTC7A* protein

We assessed expression of the *TTC7A* protein in lymphoblasts obtained from 5 patients from F1 and the patient from F2. *TTC7A* protein was not detectable in patients' T lymphoblasts, as exemplified in Fig E2, B, and barely detectable in fibroblasts from patient F5 (data not shown). These data indicate that ELA

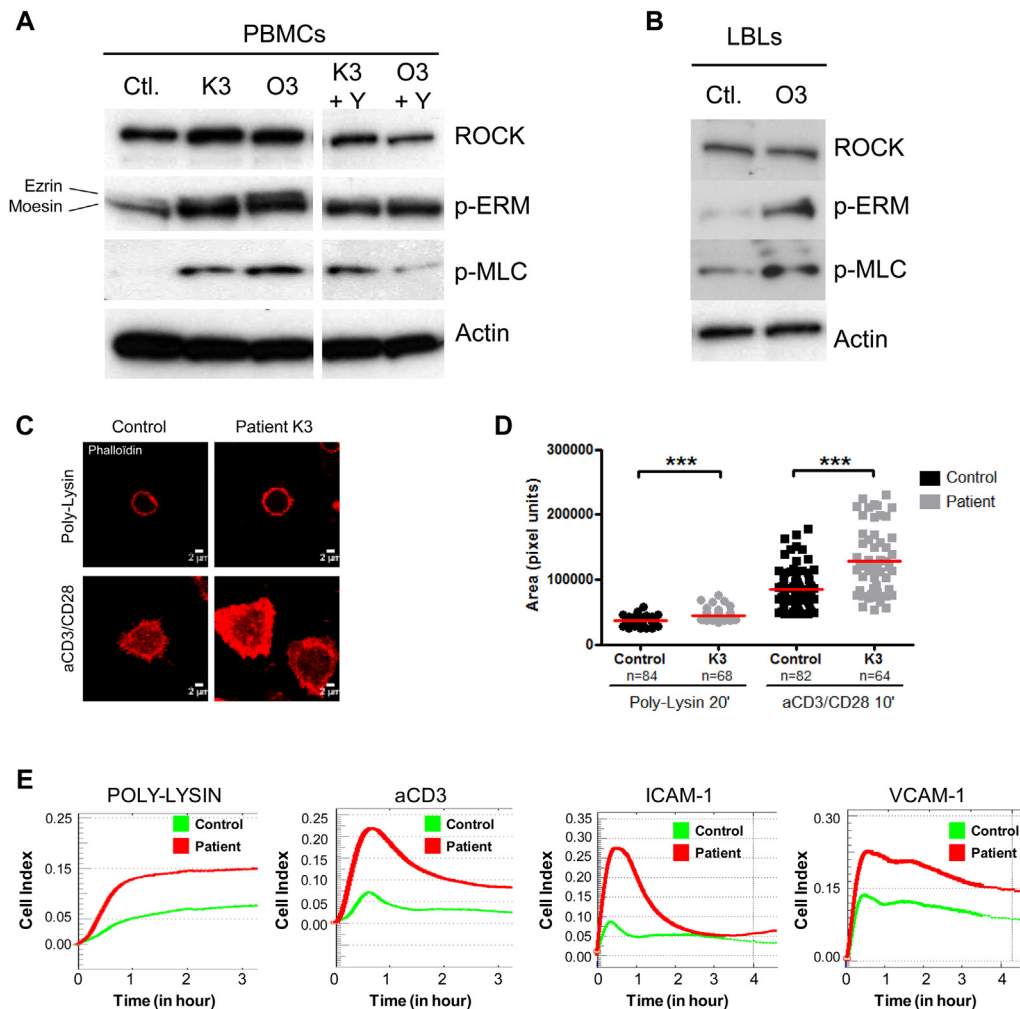


FIG 3. Increased expression of p-ERM and p-MLC and increased adhesion in *TTC7A*-deficient cells **A** and **B**, Blot showing ROCK, p-ERM, and p-MLC protein levels in whole lymphocyte lysates from a control subject and 2 patients (patients K3 and O3) before and after cell incubation with Y27632 (Fig 3, **A**) and in lymphoblastoid B cell lines (Fig 3, **B**). Similar results were observed for patient P3 from the second family. **C**, Spreading of PBMCs on polylysine- or anti-CD3/anti-CD28-coated plates. **D**, Quantification of spreading areas observed. *** $P < .0001$. **E**, Dynamic monitoring of cell adhesion of PBMCs on polylysine- or anti-CD3-, intercellular adhesion molecule 1 (*ICAM-1*)-, or vascular cell adhesion molecule 1 (*VCAM-1*)-coated plates. Similar results were obtained for 6 patients from both families.

syndrome is associated with a partial loss of *TTC7A* protein expression caused by a loss-of-function mutation in the *TTC7A* gene.

Molecular consequences of *TTC7A* deficiency

Given the perturbation of the RhoA signaling pathway observed in the gut epithelial cells of patients with null mutations in *TTC7A*,²⁴ we sought to determine whether a similar perturbation could also be detected in *TTC7A*-deficient lymphocytes from patients with ELA. We first analyzed the protein expression of RhoA kinase (ROCK) and the phosphorylation status of RhoA effector molecules (Fig 3, **A**). Whereas the ROCK protein expression was not modified, the expression of phospho-ezrin-radixin-moesin (p-ERM) and phospho-myosin light chain 2 (p-MLC) was found to be much higher in patients' PBMCs from both families and in lymphoblastoid B cell lines from 1 patient than in control cells (Fig 3, **A** and **B**, and data not shown).

ROCK is known to enhance myosin II activity by phosphorylating the regulatory myosin light chain and inhibiting myosin light chain phosphatase.²⁶ Our findings thus reflect increased ROCK activity in the patients' cells.

Functional consequences of *TTC7A* deficiency

Abnormalities related to the cytoskeleton of *TTC7A*-deficient lymphocytes. We then evaluated the consequences of RhoA signaling dysregulation on lymphocyte functionality, given that p-ERM is known to regulate the cytoskeletal rearrangements that underlie lymphocyte polarization, adhesion, and migration.²⁶ We assessed the T-cell morphologic changes and adhesion capacity, which are known to involve actomyosin dynamics. We observed that ELA T cells spread more broadly than control T cells, with an increase in the surface contact area when plated on slides coated with either polylysine or anti CD3/CD28 antibody (Fig 3, **C** and **D**).

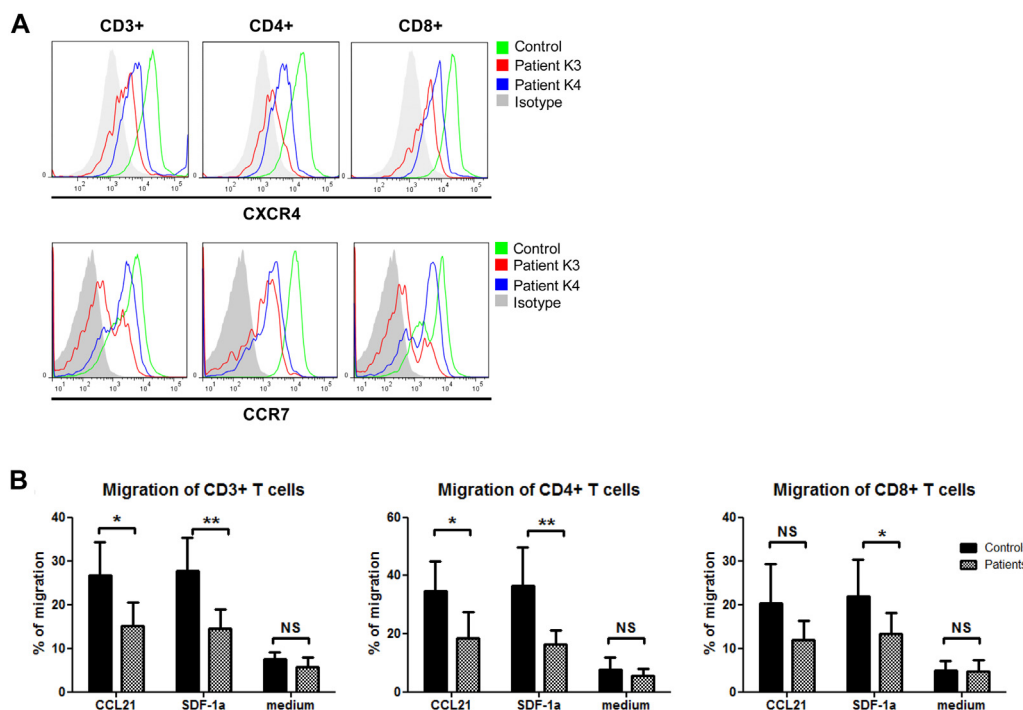


FIG 4. Impaired migration of TTC7A-deficient cells. **A**, Expression of CCR7 and CXCR4 chemokine receptors was assessed by means of flow cytometry on T-lymphocyte populations from 2 patients (patients K3 and K4; $n = 3$). **B**, PBMC migration in response to CCL21, CXCL12, or medium alone was assessed by using Transwell experiments (evaluation of the number of CD3⁺, CD4⁺, and CD8⁺ T cells that had transmigrated after 3 hours). Data are quoted as the mean $\pm$ SD assessed in triplicate in 3 independent experiments with 3 different patients. * $P < .01$ and ** $P < .001$. SDF-1a, Stromal cell-derived factor 1a; NS, not significant.

Similarly, adhesion of patients' T cells to polylysine, anti-CD3/CD28 antibodies, intercellular adhesion molecule 1, or vascular cell adhesion molecule 1 was consistently higher than adhesion of control T cells (Fig 3, E, and data not shown). Cytoskeletal remodeling is also required for cell migration. Thus we analyzed the migratory profile of TTC7A-deficient T cells. Impaired migration of TTC7A-deficient lymphocytes was observed in response to CCL21 and CXCL12 and associated with a concomitant decrease in expression of the corresponding chemokine receptors CCR7 and CXCR4 (Fig 4). Overall, these data support a role of TTC7A in the regulation of lymphocyte cytoskeletal organization and response to activation.

Increased proliferation and cell-cycle progression of TTC7A-deficient cells. TTC7A-deficient patients from family F1 had CD4 T-cell lymphocytopenia associated with preserved T-cell proliferation to mitogens but markedly impaired T-cell proliferation to antigens (see Table E1). Further assessment of the proliferation potential of TTC7A-deficient T cells was investigated in response to anti-CD3/CD28 antibody stimulation by using a sequential carboxyfluorescein succinimidyl ester dilution assay. The TTC7A-deficient T cells exhibited a significant increase in T-cell proliferation between days 1 and 3 (Fig 5, A). An increased proliferation of the TTC7A-deficient memory T cells was also observed when compared with control memory T cells (data not shown). A high fraction of patients' T cells were in the S phase at day 2 of stimulation and a corresponding lower proportion of cells were in the G1 phase when compared with those of control subjects (Fig 5, B). Similar results were obtained when other stimuli were used (PHA [Fig 5, C] or phorbol 12-myristate 13-acetate/ionomycin, data not shown). Taken as a

whole, these data suggest that the TTC7A protein is involved in the control of cell-cycle progression and T-cell proliferation.

Inhibition of ROCK rescues the TTC7A-deficient phenotype in T cells. PBMCs from patients with ELA were incubated in the presence of the synthetic ROCK inhibitor Y27632 to test whether inhibition of ROCK activity could rescue the TTC7A-deficient phenotype. In this setting patients' PBMCs indeed exhibited expression of p-ERM and p-MLC at much the same level as control PBMCs (Fig 3, A). Addition of Y27632 also inhibited the hyperproliferation of TTC7A-deficient T cells observed at day 3 after anti-CD3/CD28 stimulation (Fig 5, D). Wild-type *TTC7A* transfection in PBMCs from patients with ELA was performed to determine whether TTC7A deficiency was sufficient to account for the patients' functional defects. It restored normal cell spreading on CD3/CD28-coated plates (Fig 6). Lastly, TTC7A-deficient fibroblasts displayed overproliferation and increased p-ERM and p-MLC expressions (see Fig E3 in this article's Online Repository at www.jacionline.org), which were reduced either by a 1-hour pretreatment of the cells with Y27632 (data not shown) or by wild-type *TTC7A* transfection (see Fig E3). As expected, Y27632 had no effect on control fibroblast proliferation (data not shown). These data further support a primary role of TTC7A in several cellular functions involving cytoskeletal dynamics through the regulation of the RhoA activation pathway.

Growth and polarity impairment of gut organoids from patients with ELA

A 3-dimensional epithelial "organoid" culture^{27,28} was established from a rectum biopsy specimen from a patient with ELA

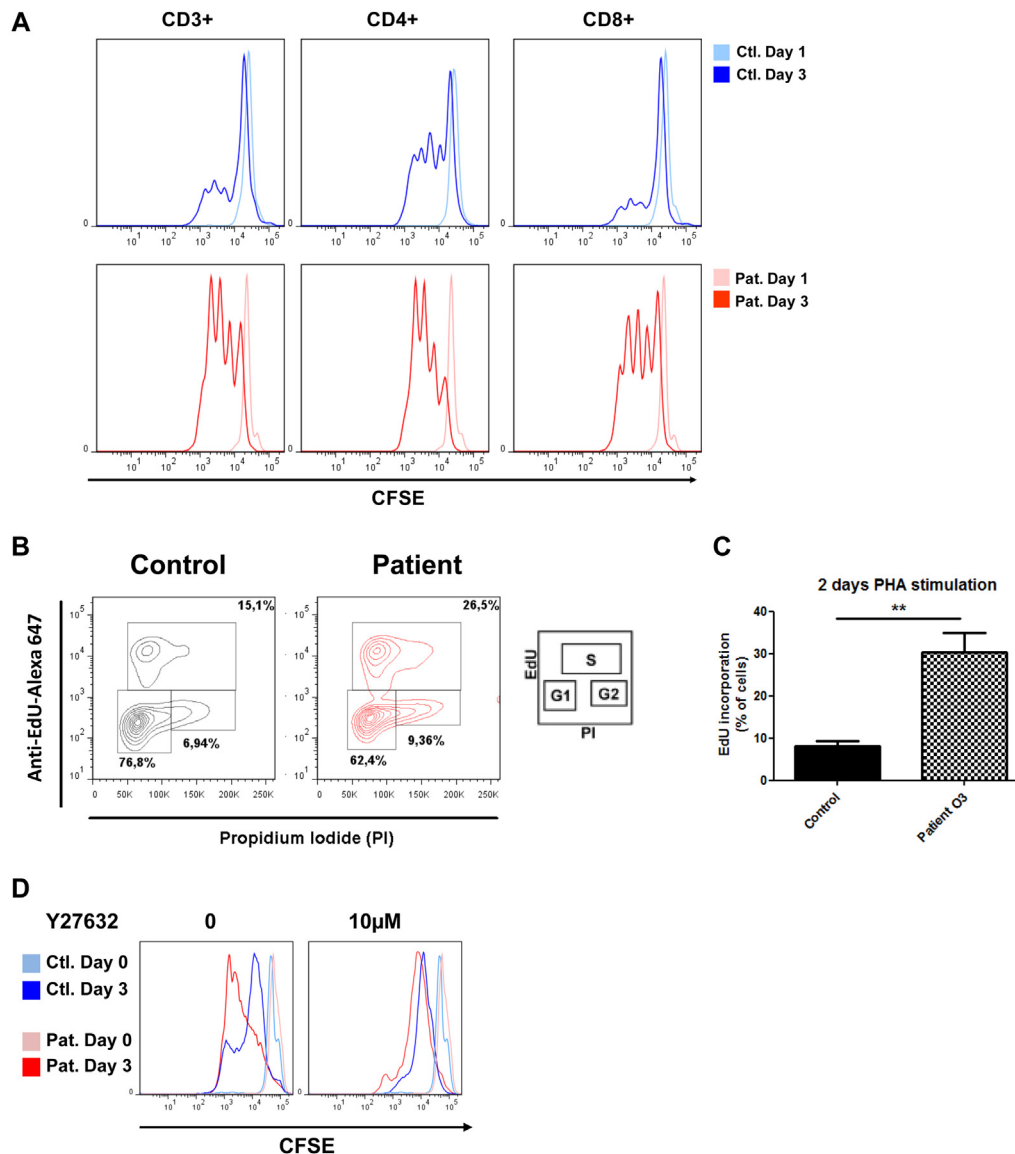


FIG 5. Increased proliferation and cell-cycle progression of TTC7A-deficient cells rescued by ROCK inhibitor. **A**, Proportions of proliferating CD3⁺, CD4⁺, and CD8⁺ T cells on day 3 after carboxyfluorescein succinimidyl ester (CFSE) labeling and anti-CD3/CD28 stimulation. Similar results were obtained for 6 patients (n = 5). **B**, Cell-cycle distribution of CD3⁺ T cells 2 days after CD3/CD28 stimulation (n = 3). **C**, Quantification of 5-ethyl-2-deoxyuridine incorporation 2 days after stimulation with PHA. *T bars* show the SE: ***P* < .001, as determined in a *t* test. **D**, Proliferation of CD3⁺ T cells in the presence of Y27632. Similar results were obtained with cells from patient R3.

(patient O3). The gut organoids displayed a disturbed epithelial architecture with condensed cell aggregates. Interestingly, continuous addition of ROCK inhibitor (Y27632) resulted in lumen expansion and restored normal expansion of the patient's organoids (Fig 7, A and B). The inhibitor was dispensable for the growth of control organoids (data not shown and Sato et al²⁸). In addition, gut "organoids" from patient O3 displayed an aberrant, partially inverted polarity, as shown by F-actin and $\alpha 6$ integrin staining (Fig 7, C and D). This anomaly was also corrected by addition of the ROCK inhibitor (Y27632; Fig 7, C and D). These data further suggest that TTC7A has a primary role in the enteropathy that characterizes the ELA phenotype.

DISCUSSION

Here we describe an autosomal recessive ELA syndrome characterized by an early onset of chronic inflammatory enteropathy and define its genetic cause. The underlying gene mutation results in loss of TTC7A expression, which was found to be associated with activation of the Rho kinase pathway. This abnormality could be corrected *in vitro* by the expression of wild-type TTC7A. The symptoms displayed by the kindred members combined features of a T-cell/B-cell immune deficiency with those of severe IBD, notably in early childhood. All the patients exhibited low numbers of memory B cells and hypogammaglobulinemia. All of them, except the patient from F2 last seen at 2 years of age, exhibited T, B, and NK lymphocytopenia and

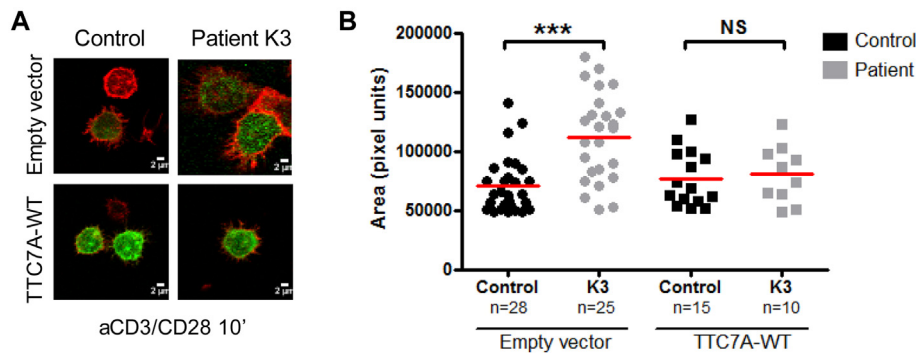


FIG 6. Rescue of the *TTC7A* phenotype by wild-type *TTC7A* (*TTC7A-WT*) expression. **A**, Merged immunofluorescence projections of PBMCs transfected with *TTC7A-WT* construct or an empty vector (in green) and labeled with phalloidin (in red). **B**, Quantification of the area of phalloidin staining observed from data analyzed blindly on 3-dimensional projections of z-stacks. Similar results were obtained for 2 patients (patients K3 and O3; $n = 2$). *** $P < .0001$. NS, Not significant.

defective antigen-specific T-cell responses *in vitro*. IBD is another key manifestation in patients with ELA syndrome and is life-threatening. It is noteworthy that despite full hematopoietic chimerism, IBD was not improved by HSCT in 1 treated subject; this finding suggests that *TTC7A* deficiency in enterocytes is a major inducer of local inflammation.

In human subjects null mutations in *TTC7A* were recently associated with a severe phenotype known as multiple intestinal atresia with severe combined immunodeficiency.²¹⁻²⁴ Since birth, all of these patients have had variable numbers of intestinal obstructions along the gastrointestinal tract and profound T, B, and NK lymphopenia and thymus hypoplasia. In mice spontaneous mutation of *ttc7* has been reported in the flaky skin mutant (*fsn*), as well as in 2 other models, the hereditary erythroblastic anemia (*hea*) and INT (*int*) mutants.²⁹⁻³² Flaky skin mice have a longer lifespan and were thus analyzed in more details. The flaky skin mouse phenotype overlaps in part with that of patients with ELA. The mice display pleiotropic skin abnormalities, with the development of thick white scales and patchy alopecia that turns into a papulosquamous disease resembling human psoriasis.^{32,33} Although only 1 adult with ELA had psoriasis, 6 had alopecia and 4 had onychopathy. Furthermore, flaky skin mice progressively have (1) massive papillomatosis of the stratified squamous epithelium of the forestomach, (2) hyperplasia and dysplasia of the glandular stomach, (3) increased apoptosis of cecal enterocytes, and (4) a moderate mixed inflammatory cell infiltrate in the lamina propria.^{34,35} These gastrointestinal changes are reminiscent of those observed in patients with ELA and in those with combined immune deficiency, with patients with multiple intestinal atresia carrying a null mutation in *TTC7A*.²⁴ These epithelial anomalies further suggest that *TTC7A* plays a key role in gut intestinal epithelial cell development and maintenance. In addition, flaky skin mice exhibit a hyperproliferative immune disorder combining splenomegaly, lymphadenopathy (accumulated T and B lymphocytes), increased proliferation in response to mitogens, and features of systemic autoimmunity that persist over time. Although the immunologic phenotype is not the same in murine and human *TTC7A* natural mutants, there are common features. Both murine and human phenotypes display excessive T-lymphocyte proliferation and an impaired cellular response to antigens. Mouse versus human disparities in phenotypic expression might be related to the time at which the assessment is carried out, the

nature of the mutation, the disease's environment, and, of course, species-specific differences.

The function of the *TTC7A* protein is unknown. It contains 9 cognate tetratricopeptide repeat (TPR) domains, which might be involved in multiple protein-protein interactions. Disorders resulting from mutations in genes that encode TPR-containing proteins have been reported, including tricohepatoenteric syndrome, which is also characterized by the presence of a protracted gut disease.³⁶ Nevertheless, relatively little is known about the function of proteins that contain TPR domains or the pathogenesis of conditions in which these proteins are defective.

We previously established that null mutations in *TTC7A* disrupt intestinal epithelial polarity and increase RhoA activity in patients with multiple intestinal atresias and severe combined immune deficiency.²⁴ Here we show that *TTC7A* deficiency resulting from hypomorphic mutations in the gene impairs the RhoA signaling pathway in a way that does not hamper intestinal development but partially impairs epithelial cell polarity and gut homeostasis. In addition, we show that in hematopoietic cells an inappropriate activation of the RhoA signaling pathway is also detected, a pathway that is known to orchestrate the interplay between the plasma membrane and the cortical cytoskeleton.³⁷ In particular, *TTC7A* deficiency induces the phosphorylation of several ROCK effectors (eg, ezrin-radixin-moesin and myosin light chain) that are known to regulate cell adhesion, polarization, and migration.^{26,38,39} Accordingly, T lymphocytes from patients with ELA exhibit multiple alterations related to cytoskeletal remodeling, such as increased spreading, adhesion, and reduced capacity of migration in response to a range of chemoattractants. Remodeling of the cytoskeleton in T and dendritic cells is known to play a critical role in the organization of the immunologic synapse and thus in T-cell proliferation/activation or effector functions, which might account for the panlymphopenia observed.⁴⁰ This role is exemplified in another natural model of cytoskeletal dysfunction (ie, deficiency of the Wiskott-Aldrich syndrome protein, which impairs T-cell priming).⁴¹

Hence *TTC7A* deficiency, through defective control of RhoA signaling pathway activation, modulates the proliferation capacity of lymphocytes. However, the mechanism by which *TTC7A* regulates the ROCK pathway remains to be established. A role of *TTC7A* deficiency in thymic epithelial function might also contribute to the immune disorder because thymic epithelial cells express *TTC7A*²³ and distribution of thymic epithelial cells

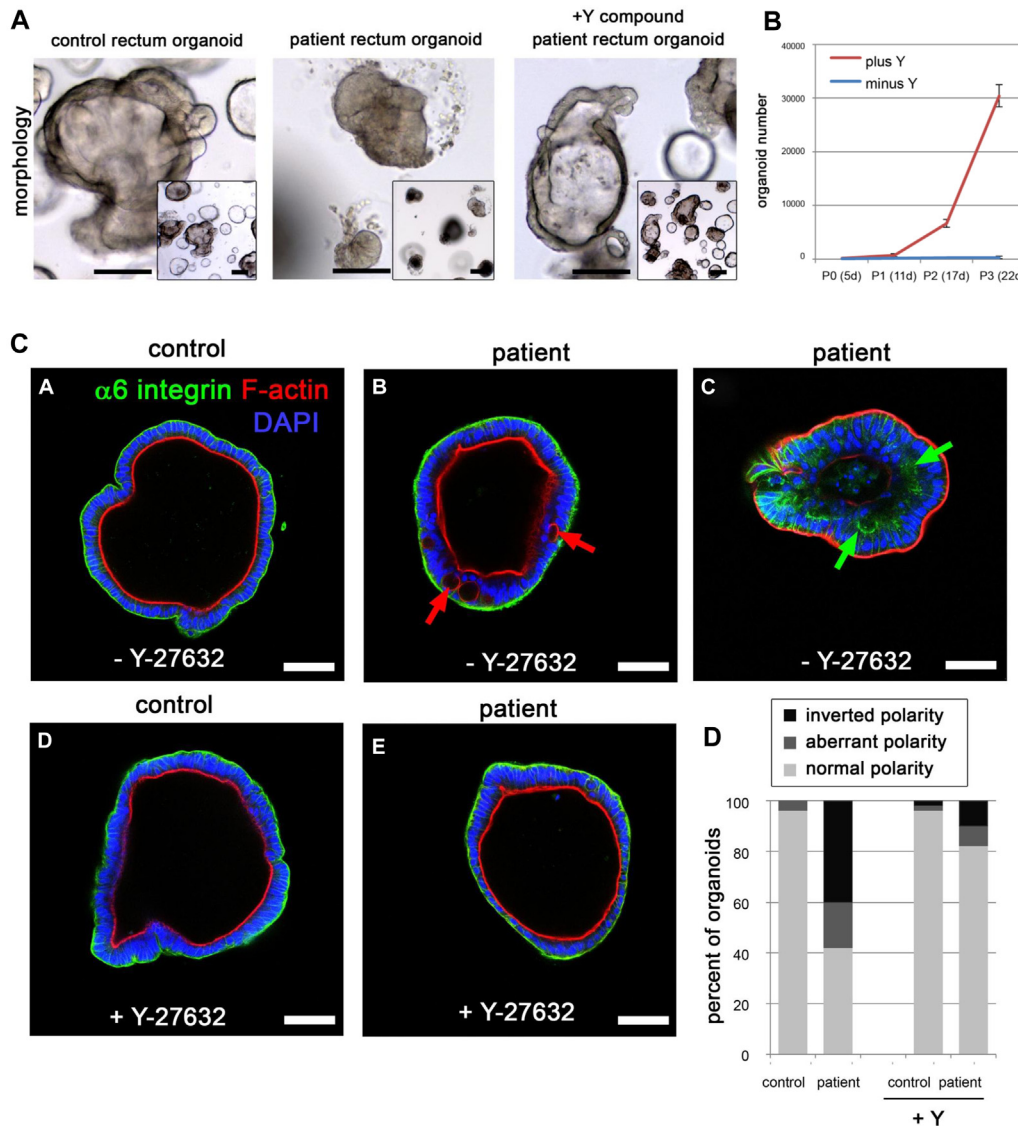


FIG 7. ROCK inhibition normalizes growth and apicobasal polarity in patients' organoids. **A** and **B**, Morphologic images (Fig 7, A) and growth curve (Fig 7, B) of human rectal organoids from a healthy control subject and from patient O3 incubated in the presence or absence of 10 $\mu\text{mol/L}$ Y27632 ($n = 3$). **C**, Immunofluorescence staining of $\alpha 6$ -integrin (green) and F-actin (red) and nuclei with 4'-6-diamidino-2-phenylindole dihydrochloride (DAPI; blue) at day 5 of culture. Aberrant polarity is characterized by F-actin deposition around vacuoles (red arrows). Inverted polarity is characterized by F-actin deposition on the outer side and $\alpha 6$ -integrin staining on the inner face of the epithelium (green arrows). Scale bars = 50 μm . **D**, We scored the percentages of organoids with normal, aberrant, and inverted polarity. Fifty organoids were counted for each condition.

was affected in a patient with null mutation in *TTC7A*.²⁴ Recently, very early-onset IBD with similar histologic features was reported in 3 patients carrying a missense mutation in *TTC7A*.²¹ Lymphopenia was noticed in 1 patient, but no other features (alopecia and autoimmune manifestations) were seen in these patients up to age 14 months. *TTC7A* was shown to interact with EFR3B and PI4KIIIa and modulate phosphatidylinositol 4-phosphate levels at the plasma membrane.^{21,42} It will be interesting to decipher how these interactions, as well as potentially others, account for the *TTC7A*-mediated control of the Rho kinase pathway and whether *TTC7A* exerts effects on additional pathways.

Our study further extends the spectrum of *TTC7A*-associated disease ranging from multiple intestinal atresia with severe

combined immune deficiency to early-onset IBD with a variable degree of immune deficiency. In some cases alopecia is an additional diagnostic feature of this clinical entity. Moreover, we show a role of *TTC7A* in lymphocytes that contributes to the regulation of cell proliferation, adhesion, and migration. We show that *TTC7A* mutation directly affects both lymphocyte and epithelial cell functions in association with an increase in ROCK activity. The fact that this increase can be reverted *in vitro* by treatment with a ROCK inhibitor suggests that this ELA syndrome is amenable to pharmacologic manipulation.

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Key messages

- **TTC7A regulates the proliferation, adhesion, and migration of lymphocytes in addition to the polarity of epithelial cells by controlling RhoA signaling pathway activation.**
- **This condition appears as the first in which impairments of both lymphocyte and epithelial cell functions contribute to an IBD.**
- **Our findings suggest that administration of a Rho kinase pathway inhibitor could be considered as a treatment of this form of IBD and that perhaps the concept could be extended to more common forms of IBDs.**

REFERENCES

- Gismera CS, Aladren BS. Inflammatory bowel diseases: a disease (s) of modern times? Is incidence still increasing? *World J Gastroenterol* 2008;14:5491-8.
- Abraham C, Cho JH. Inflammatory bowel disease. *N Engl J Med* 2009;361:2066-78.
- Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 2007;448:427-34.
- Sartor RB. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. *Nat Clin Pract Gastroenterol Hepatol* 2006;3:390-407.
- Baumgart DC, Sandborn WJ. Crohn's disease. *Lancet* 2012;380:1590-605.
- Brandl K, Beutler B. Creating diseases to understand what prevents them: genetic analysis of inflammation in the gastrointestinal tract. *Curr Opin Immunol* 2012;24:678-85.
- Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci U S A* 2007;104:13780-5.
- Nenci A, Becker C, Wullaert A, Gareus R, van Loo G, Danese S, et al. Epithelial NEMO links innate immunity to chronic intestinal inflammation. *Nature* 2007;446:557-61.
- Iqbal N, Oliver JR, Wagner FH, Lazenby AS, Elson CO, Weaver CT. T helper 1 and T helper 2 cells are pathogenic in an antigen-specific model of colitis. *J Exp Med* 2002;195:71-84.
- Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 2012;491:119-24.
- Lapaquette P, Brest P, Hofman P, Darfeuille-Michaud A. Etiology of Crohn's disease: many roads lead to autophagy. *J Mol Med (Berl)* 2012;90:987-96.
- Franke A, McGovern DP, Barrett JC, Wang K, Radford-Smith GL, Ahmad T, et al. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. *Nat Genet* 2010;42:1118-25.
- Anderson CA, Boucher G, Lees CW, Franke A, D'Amato M, Taylor KD, et al. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. *Nat Genet* 2011;43:246-52.
- Budarf ML, Labbe C, David G, Rioux JD. GWA studies: rewriting the story of IBD. *Trends Genet* 2009;25:137-46.
- Cooney R, Baker J, Brain O, Danis B, Pichulik T, Allan P, et al. NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. *Nat Med* 2010;16:90-7.
- Glocker EO, Kotlarz D, Boztug K, Gertz EM, Schaffer AA, Noyan F, et al. Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. *N Engl J Med* 2009;361:2033-45.
- Glocker EO, Frede N, Perro M, Sebire N, Elawad M, Shah N, et al. Infant colitis—it's in the genes. *Lancet* 2010;376:1272.
- Pachlopnik Schmid J, Canioni D, Moshous D, Touzot F, Mahlaoui N, Hauck F, et al. Clinical similarities and differences of patients with X-linked lymphoproliferative syndrome type 1 (XLP-1/SAP deficiency) versus type 2 (XLP-2/XIAP deficiency). *Blood* 2011;117:1522-9.
- Krieg A, Correa RG, Garrison JB, Le Negrato G, Welsh K, Huang Z, et al. XIAP mediates NOD signaling via interaction with RIP2. *Proc Natl Acad Sci U S A* 2009;106:14524-9.
- Schappi MG, Jaquet V, Belli DC, Krause KH. Hyperinflammation in chronic granulomatous disease and anti-inflammatory role of the phagocyte NADPH oxidase. *Semin Immunopathol* 2008;30:255-71.
- Avitzur Y, Guo C, Mastropaolo LA, Bahrami E, Chen H, Zhao Z, et al. Mutations in tetratricopeptide repeat domain 7A result in a severe form of very early onset inflammatory bowel disease. *Gastroenterology* 2014;146:1028-39.
- Samuels ME, Majewski J, Alirezaie N, Fernandez I, Casals F, Patey N, et al. Exome sequencing identifies mutations in the gene TTC7A in French-Canadian cases with hereditary multiple intestinal atresia. *J Med Genet* 2013;50:324-9.
- Chen R, Giliani S, Lanzi G, Mias GI, Lonardi S, Dobbs K, et al. Whole-exome sequencing identifies tetratricopeptide repeat domain 7A (TTC7A) mutations for combined immunodeficiency with intestinal atresias. *J Allergy Clin Immunol* 2013;132:656-64.e17.
- Bigorgne AE, Farin HF, Lemoine R, Mahlaoui N, Lambert N, Gil M, et al. TTC7A mutations disrupt intestinal epithelial apicobasal polarity. *J Clin Invest* 2014;124:328-37.
- Wu C, Orozco C, Boyer J, Leglise M, Goodale J, Batalov S, et al. BioGPS: an extensible and customizable portal for querying and organizing gene annotation resources. *Genome Biol* 2009;10:R130.
- Schmitz AA, Govek EE, Bottner B, Van Aelst L. Rho GTPases: signaling, migration, and invasion. *Exp Cell Res* 2000;261:1-12.
- Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* 2009;459:262-5.
- Sato T, Stange DE, Ferrante M, Vries RG, Van Es JH, Van den Brink S, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology* 2011;141:1762-72.
- White RA, McNulty SG, Roman S, Garg U, Wirtz E, Kohlbrecher D, et al. Chromosomal localization, hematologic characterization, and iron metabolism of the hereditary erythroblastic anemia (hea) mutant mouse. *Blood* 2004;104:1511-8.
- White RA, McNulty SG, Nsumu NN, Boydston LA, Brewer BP, Shimizu K. Positional cloning of the Ttc7 gene required for normal iron homeostasis and mutated in hea and fsn anemia mice. *Genomics* 2005;85:330-7.
- Helms C, Pelsue S, Cao L, Lamb E, Loffredo B, Taillon-Miller P, et al. The Tetratricopeptide repeat domain 7 gene is mutated in flaky skin mice: a model for psoriasis, autoimmunity, and anemia. *Exp Biol Med (Maywood)* 2005;230:659-67.
- Takabayashi S, Iwashita S, Hirashima T, Katoh H. The novel tetratricopeptide repeat domain 7 mutation, Ttc7^{fsn}-Jic, with deletion of the TPR-2B repeat causes severe flaky skin phenotype. *Exp Biol Med (Maywood)* 2007;232:695-9.
- Sundberg JP, Oliver RF, McElwee KJ, King LE Jr. Alopecia areata in humans and other mammalian species. *J Invest Dermatol* 1995;104(suppl):32S-3S.
- Sundberg JP, France M, Boggess D, Sundberg BA, Jensen AB, Beamer WG, et al. Development and progression of psoriasiform dermatitis and systemic lesions in the flaky skin (fsn) mouse mutant. *Pathobiology* 1997;65:271-86.
- Sundberg JP, Kenty GA, Beamer WG, Adkison DL. Forestomach papillomas in flaky skin and steel-Dickie mutant mice. *J Vet Diagn Invest* 1992;4:312-7.
- Hartley JL, Zachos NC, Dawood B, Donowitz M, Forman J, Pollitt RJ, et al. Mutations in TTC37 cause trichohepatoenteric syndrome (phenotypic diarrhea of infancy). *Gastroenterology* 2010;138:2388-98, e1-2.
- de Curtis I, Meldolesi J. Cell surface dynamics—how Rho GTPases orchestrate the interplay between the plasma membrane and the cortical cytoskeleton. *J Cell Sci* 2012;125:4435-44.
- Spiering D, Hodgson L. Dynamics of the Rho-family small GTPases in actin regulation and motility. *Cell Adh Migr* 2011;5:170-80.
- Jacobelli J, Bennett FC, Pandurangi P, Tooley AJ, Krummel MF. Myosin-IIA and ICAM-1 regulate the interchange between two distinct modes of T cell migration. *J Immunol* 2009;182:2041-50.
- Sims TN, Soos TJ, Xenias HS, Dubin-Thaler B, Hofman JM, Waite JC, et al. Opposing effects of PKC θ and WASp on symmetry breaking and relocation of the immunological synapse. *Cell* 2007;129:773-85.
- Bouma G, Mendoza-Naranjo A, Blundell MP, de Falco E, Parsley KL, Burns SO, et al. Cytoskeletal remodeling mediated by WASp in dendritic cells is necessary for normal immune synapse formation and T-cell priming. *Blood* 2011;118:2492-501.
- Nakatsu F, Baskin JM, Chung J, Tanner LB, Shui G, Lee SY, et al. PtdIns4P synthesis by PI4KIII α at the plasma membrane and its impact on plasma membrane identity. *J Cell Biol* 2012;199:1003-16.

METHODS

Study design

Clinical information and blood samples were collected from the patients, their relatives, and control subjects, all of whom had given prior informed consent to participation 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.

SNP linkage analysis and sequence analysis

SNP, sequence analysis, and exome sequencing analysis were performed, as described elsewhere.^{E1,E2}

Immunohistochemistry

Paraffin-embedded sections were stained with hematoxylin and eosin. For detection of lymphocytes, plasma cells, and macrophages, monoclonal mouse anti-CD8 (C8/144B, 1:100; Dako, Glostrup, Denmark), anti-CD4 (4B12, 1:100; Thermo Fisher Scientific, Waltham, Mass), anti-CD20 (L26, 1:400; Dako), anti-CD138 (MI15, 1:50; Dako), and anti-CD68 (KP1, 1:3000; Dako) were used, and sections were developed, as previously described.^{E3}

Cells and cell cultures

PBMCs, B-lymphoblast cell lines transformed with EBV, and fibroblast cell lines were prepared, as described elsewhere.^{E2} Lymphoblasts were obtained by incubating PBMCs for 72 hours with PHA (1:400 dilution; Sigma-Aldrich, St Louis, Mo) and IL-2 (50 IU/mL; PeproTech, Rocky Hill, NJ) in Panserin medium (PAN-Biotech GmbH, Aidenbach, Germany) supplemented with 10% human AB serum (Etablissement Français du Sang, La Plaine Saint-Denis, France). We then added more IL-2 (100 IU/mL) and cultured the cells for 6 to 7 days.

Three-dimensional intestinal organoid cultures

Biopsy specimens were manually dissected, and the mucosal layer was cut into small pieces that were washed, embedded in Matrigel (BD Biosciences, Le Pont de Claix, France), and cultured in conditions, as previously described.^{E4} The ROCK inhibitor Y27632 (10 μ M/L; Sigma) was added during establishment and maintenance of patient cultures, as indicated. Organoids were derived from the rectum of patient O3. Two independent “healthy control” cultures each were analyzed in parallel. Organoid stainings were performed, as previously described.^{E4}

Gene expression analysis

Quantitative PCR was performed on cDNA by using TaqMan PCR Master Mix (Applied Biosystems, Foster City, Calif) and *TTC7A*- and actin-specific primers. Fluorescence during PCR was measured on an ABI 7900 cyclor and analyzed with Sequence Detection Systems software (version 2.2.2, Applied Biosystems).

Protein blotting

Cell extracts and protein blots were prepared, as described elsewhere.^{E1} Antibodies against anti-ROCK, anti-p-ERM, and anti-p-MLC (from Cell Signaling, Danvers, Mass), horseradish-peroxidase-conjugated secondary antibody, and polyclonal antibody against human *TTC7A* prepared by immunizing rabbits with a *TTC7A*-GST fusion protein and then using affinity purification (from Agrobio, La-Ferté-Saint-Aubin, France) were used.

Flow cytometry

Blood lymphocytes were stained with PeCy7-conjugated anti-CCR7 and phycoerythrin-conjugated anti-CXCR4. Data were collected on a FACSCanto system and analyzed with FlowJo 8.8.4 software (TreeStar, Ashland, Ore).

Proliferation assays

PBMCs from patients and control subjects were purified by means of density gradient centrifugation and cultured for 3 days with PHA (5 μ g/mL) and for 5 days with tetanus toxoid antigen (0.125 IU/mL; Statens Serum Institute, Copenhagen, Denmark). Tritiated thymidine was added for the last 18 hours. Cell proliferation was determined as the counts per minute of tritiated thymidine incorporated. Proliferation was monitored by labeling T cells with 10 μ M/L carboxyfluorescein succinimidyl ester (Invitrogen, Carlsbad, Calif) before stimulation with anti-CD3/CD28 beads (Invitrogen), according to the manufacturer's instructions.

Cell-cycle analysis

Cell-cycle analyses were performed by means of incorporation of the nucleoside analogue 5-ethyl-2-deoxyuridine into newly synthesized DNA 2 or 5 days after stimulation with anti-CD3/CD28 beads or PHA, according to the manufacturer's instructions (Click-itEdU, Invitrogen).

Spreading area analysis

Freshly isolated PBMCs (1×10^5) were seeded onto polylysine with or without anti-CD3/anti-CD28-coated cover slips. Cells were allowed to adhere and spread at different time points at 37°C. Adherent cells were fixed in 4% paraformaldehyde for 15 minutes, followed by staining for F-actin (Alexa Fluor 555-conjugated anti-rabbit phalloidin). The cells were then analyzed with the LSM 700 machine (Carl Zeiss, Oberkochen, Germany) for confocal microscopy. The spreading cell area was calculated from the circumference on 3-dimensional projections of z-stacks of phalloidin-labeled cells by using the ImageJ software “Analysis particles” function.

Chemotaxis assay

Chemotaxis assays were performed with Transwell chambers (5- μ m pore size) with 200 μ L of cell suspension (1×10^6 cells/mL) in the top chamber and 600 ng/mL CCL21 or 1 μ g/mL CXCL12 in the bottom chamber. After 3 hours at 37°C in 5% CO₂, the proportion of migrated cells was calculated by using flow cytometry.

Cell adhesion and proliferation assays

Cell adhesion and proliferation assays were done with xCELLigence technology (Roche, Mannheim, Germany). The E-plate wells were coated with polylysine in the presence or absence of anti-CD3, intercellular adhesion molecule 1, or vascular cell adhesion molecule 1. Adhesion of PBMCs was automatically monitored every 10 seconds over 4 hours. For the proliferation assay, 1500 fibroblasts transfected with an empty vector pEGFP-C1 or wild-type *TTC7A*-pEGFP tagged vector were added directly to E-plate wells. Lastly, cell proliferation was monitored every 10 minutes for up to 147 hours. Electrical impedance was measured by using the xCELLigence system's integrated software and expressed as a cell index.

Rescue experiments

Full-length *TTC7A* cDNA was subcloned, as previously described.^{E3} Restoration of the normal phenotype of *TTC7A*-deficient cells was evaluated with fibroblasts and freshly isolated PBMCs transfected with an empty vector pEGFP-C1 or a wild-type *TTC7A*-pEGFP tagged vector, according to the manufacturer's instructions. Western blotting, xCELLigence experiments, and confocal microscopy were performed 24 hours after transfection.

Statistical analysis

Analyses were performed with Prism software (version 4 for Macintosh; GraphPad Software, La Jolla, Calif). Statistical hypotheses were analyzed with *t* tests and Mann-Whitney *U* tests.

Accession codes

The GenBank accession code for *TTC7A* was NM_020458.2.

REFERENCES

- E1. Cote M, Menager MM, Burgess A, Mahlaoui N, Picard C, Schaffner C, et al. Munc18-2 deficiency causes familial hemophagocytic lymphohistiocytosis type 5 and impairs cytotoxic granule exocytosis in patient NK cells. *J Clin Invest* 2009;119:3765-73.
- E2. Pachlopnik Schmid J, Lemoine R, Nehme N, Cormier-Daire V, Revy P, Debeurme F, et al. Polymerase epsilon1 mutation in a human syndrome with facial dysmorphism, immunodeficiency, livedo, and short stature ("FILS syndrome"). *J Exp Med* 2012;209:2323-30.
- E3. Bigorgne AE, Farin HF, Lemoine R, Mahlaoui N, Lambert N, Gil M, et al. TTC7A mutations disrupt intestinal epithelial apicobasal polarity. *J Clin Invest* 2014;124:328-37.
- E4. Sato T, Stange DE, Ferrante M, Vries RG, Van Es JH, Van den Brink S, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology* 2011;141:1762-72.

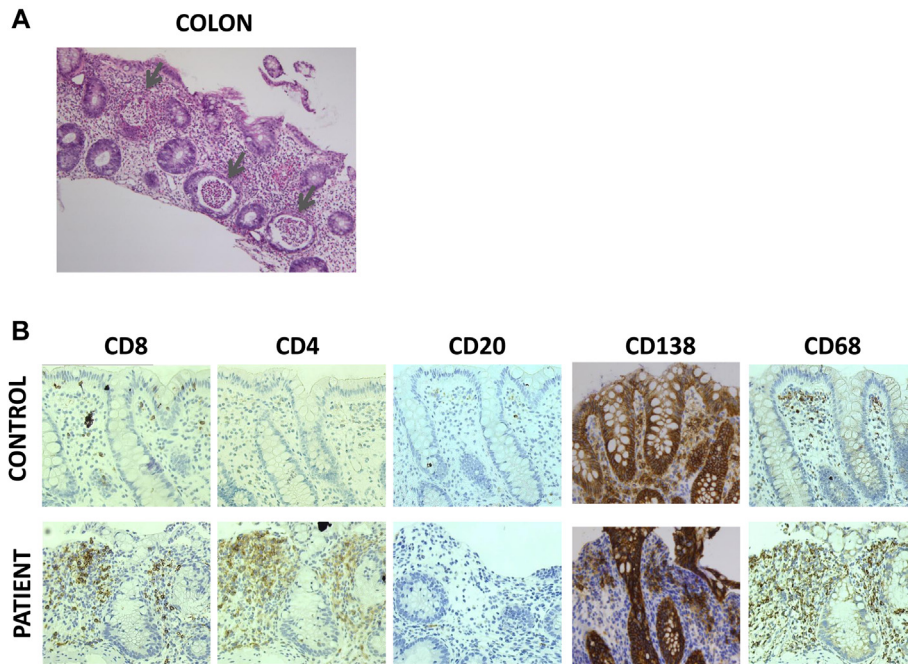


FIG E1. Inflammatory infiltrate of the gut in patients with ELA. **A**, Colon sample from patient K4, with multiple cryptic abscesses indicated by *arrows* (magnification $\times 100$). **B**, Phenotypic characterization of the inflammatory infiltrate in the lamina propria of the colon (patient K4; magnification $\times 200$).

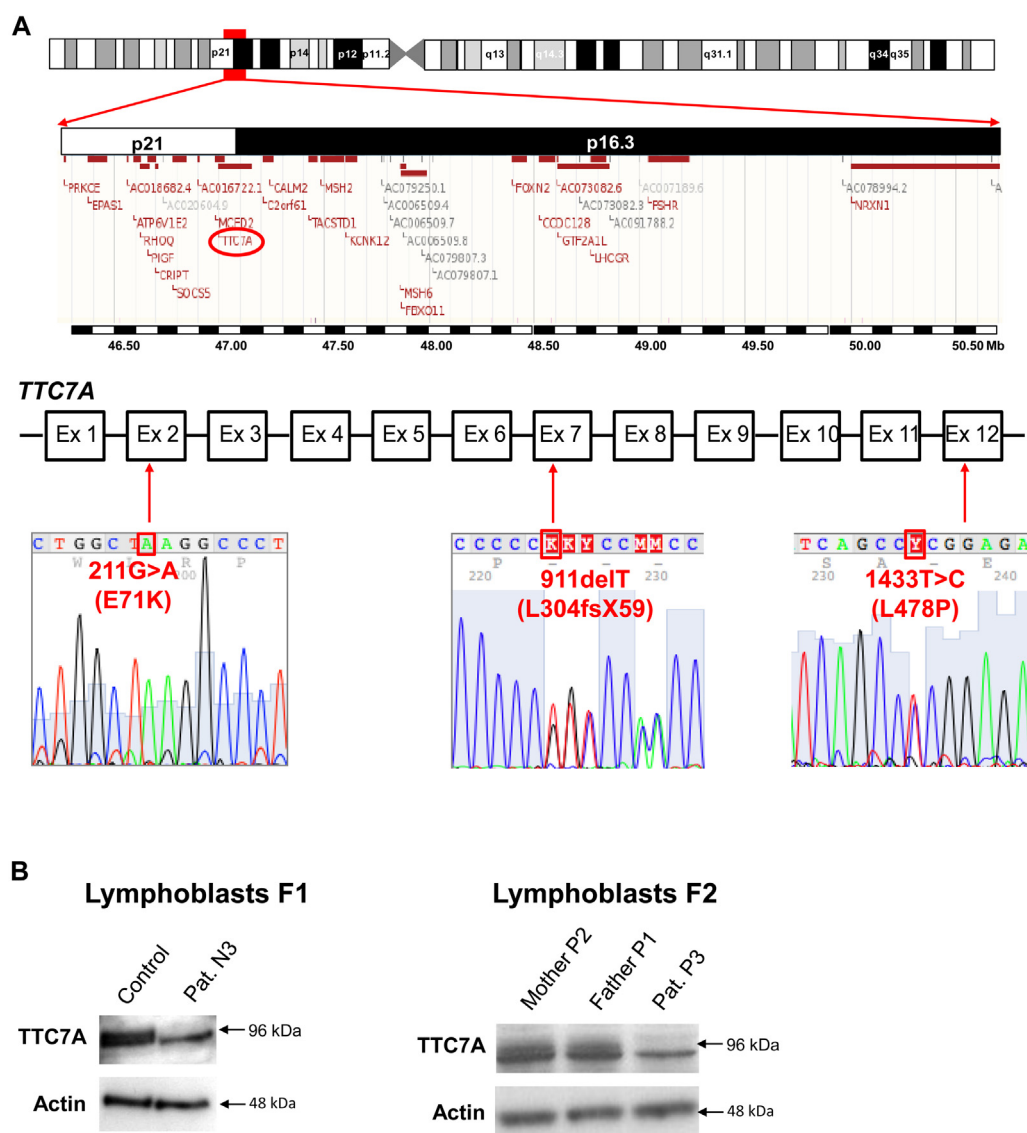


FIG E2. Mutations in *TTC7A* and defective expression of *TTC7A* protein. **A**, Schematic representation of the 4-Mb candidate interval on the short arm of chromosome 2 extending from base pair 46,321,800 to base pair 50,570,974. The candidate region contains 23 putative protein-coding genes, including *TTC7A* (red circle). The different mutations identified in *TTC7A* exons 2, 7, and 12 are shown. **B**, Western blot analysis of *TTC7A* protein expression in lymphoblasts from a healthy control subject and patient N3 from family F1 (left) and from the parents (P1 and P2) and a patient (P3) from family F2 (right; n = 6). Actin was used as a loading control.

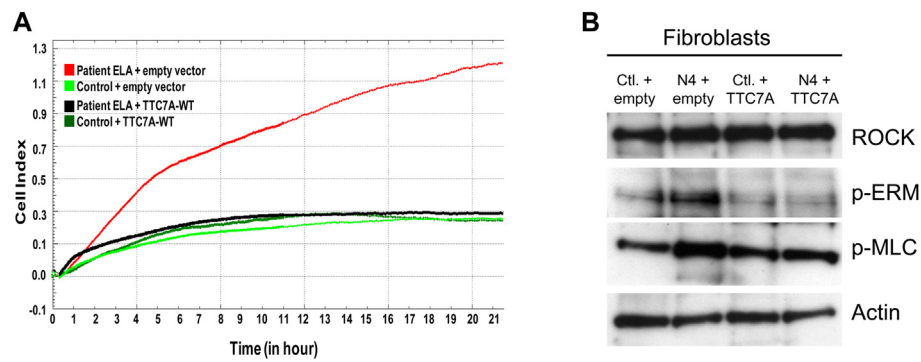


FIG E3. Rescue of the *TTC7A* phenotype in fibroblasts by wild-type *TTC7A* (*TTC7A-WT*) expression. **A** and **B**, Patient-derived fibroblasts were transiently transfected with a *TTC7A-WT* construct or an empty vector before adhesion measurement followed by proliferation by using xCELLigence technology (Fig E3, A) and before ROCK, p-ERM, and p-MLC expression analysis by using Western blotting 24 hours later (Fig E3, B; n = 3).

TABLE E1. Immunologic features of patients with IBD

Patient ID*	R3	Control values	N3	F5	K4	K3	N4	P3	Control values	O3	D8	Control values	A3	B4	C1	Control values
Age (y)	4/12	(3-5)/12	6/12	6/12	7/12	9/12	10/12	12/12	(6-12)/12	2	4	2-6	14	28	50	12-adult
Lymphocytes (per $\mu\text{L} \times 10^{-3}$)	2.9	3.9-9.0	1.9	2.2	2.8	1.9	1.4	5.3	3.4-9.0	2.8		2.3-5.4	0.7	0.5	0.80	1.4-3.3
T cells																
CD3 ⁺ (per $\mu\text{L} \times 10^{-3}$)	2.3	2.5-5.6	1.1	2.0	1.7	1.4	0.7	4.5	1.9-5.9	1.7	0.1	1.4-3.7	0.6	0.3	0.67	1.0-2.2
CD4 ⁺ (%)	51	35-56	23	40	26	0.5	23	56	31-56	21	6	28-47	36	25	49	31-52
CD4 ⁺ CD45RA ⁺ CD31 ⁺ naive	33	60-72	35		63	71	47	57	60-72	47		50-85	11	11	2	43-55
CD4 ⁺ CD45RO ⁺	67	6-23	40		27		45	7	7-36	35		14-47	88	85	94	34-67
CD8 ⁺ (%)	25	12-23	33	35	30	0.8	19	24	12-24	33	3	16-30	57	35	25	18-35
CD8 ⁺ CD45RA ⁺ CCR7 ⁺ naive	49	52-68	93				81	86	52-68	28		52-68	6	6	0.25	30-50
CD8 ⁺ CD45RA ⁺ CCR7 ⁻	16	16-28	4				5	7	16-28	65		16-28	92	27	9	8-20
CD8 ⁺ CD45RA ⁻ CCR7 ⁺	1	3-4	1				4	3	3-4	0.6		3-4	0	2	6	6-16
CD8 ⁺ CD45RA ⁻ CCR7 ⁻	33	11-20	2				10	4	11-20	8		11-20	2	65	85	20-40
Treg CD4 ⁺ CD25 ^{hi} FoxP3 ⁺ (%)	0.7	2-6			2.6	1.4		1.5	3.5-8	1.1		2.5-6	0.4	1.1		2.8-4.2
T-cell proliferation (cpm $\times 10^{-3}$)																
PHA	48	>50	30	116	205	86	104	89	>50	74	38	>50	31	36	4	>50
CD3	6	>30		78				ND	>30	42	ND	>30		44	10	>30
Tetanus toxoid	ND		ND		1	0	1.5	16	>10	8	44	>10	1	8	1	>10
CMV	0.3	>10	ND	2			0	ND	>10	0.5	1	>10	16.5	0.9		>10
Candida species antigen	1	>10	15	1	0	0	1	0.85	>10	1	73	>10		7.5	5	>10
NK cells																
CD56 ⁺ CD16 ⁺ (per $\mu\text{L} \times 10^{-3}$)	0.09	0.2-0.8	0.06		0.05	0.02	0.04	0.21	0.2-0.9	0.06	0.01	0.2-0.9	0.01	0.01	0.11	0.07-0.58
B cells																
CD19 ⁺ (per $\mu\text{L} \times 10^{-3}$)	0.46	0.43-3.0	0.89	0.29	1.1	0.5	0.7	0.74	0.6-2.6	1.0	0.05	0.7-2.6	0.04	0.09	0	0.09-0.57
CD27 ⁺ /CD19 ⁺ memory (%)	3	7-27	2		1.5	3		3	7-27	2		10-27	5	4	0	24-45
Immunoglobulin levels (g/L)																
IgM	0.14	0.34-0.95	0.31	0.06	<0.04	0.32	ND	<0.22	0.4-1.0	<0.05	0.26	0.5-1.4	0.1	0.17	0.19	0.5-2.1
IgG	2.49	2.35-4.37	1.7	1.3	2.6	1.5	ND	6.44	2.3-6.23	6.08	2	4.0-7.5	2	8.97	3.7	6.6-12.3
IgA	0.08	0.20-0.62	0.07	<0.07	0.08	0.22	ND	0.02	0.2-0.8	0.25	0.12	0.3-1.1	0.48	2.3	0.15	1.16-2.6

FoxP3, Forkhead box protein 3; ND, not done; Treg, regulatory T.

*Patients B3 and E3 lack a detailed immunologic analysis and thus were not included in the table.