OPEN

Multiple Intestinal Atresia With Combined Immune Deficiency Related to TTC7A Defect Is a Multiorgan Pathology

Study of a French-Canadian-Based Cohort

Isabel Fernandez, PhD, Natalie Patey, MD, PhD, Valérie Marchand, MD, Mirela Birlea, Bsc, Bruno Maranda, MD, Elie Haddad, MD, PhD, Hélène Decaluwe, MD, PhD, and Françoise Le Deist, MD, PhD

Abstract: Hereditary multiple intestinal atresia (HMIA) is a rare cause of intestinal obstruction in humans associated with a profound combined immune deficiency. Deleterious mutations of the tetratricopeptide repeat domain–7A (*TTC7A*) gene lead to HMIA, although the mechanism(s) causing the disease in TTC7A deficiency has (have) not yet been clearly identified.

To evaluate the consequences of TTC7A deficiency, we studied the morphology of several organs from HMIA patients at different developmental stages, as well as the expression of the TTC7A protein. We performed histological and immunohistochemical analyses on biopsies and autopsies of 6 patients and 1 fetus with HMIA. Moreover, we characterized for the first time the expression of the TTC7A protein by immunostaining it in several organs from control (including fetal samples), infants, and 1 fetus with HMIA.

Besides the gastrointestinal tract, HMIA disease was associated with morphological alterations in multiple organs: thymus, lung, spleen, and liver. Moreover, we demonstrated that normal TTC7A protein was expressed in the cytoplasm of epithelial cells of the intestine, thymus, and pancreas. Surprisingly, altered TTC7A protein was highly expressed in tissues from patients, mainly in the epithelial cells.

We have established that HMIA associated with a TTC7A defect is characterized by multiorgan impairments. Overall, this report suggests that TTC7A protein is critical for the proper development, preservation, and/or function of thymic and gastrointestinal epithelium.

(*Medicine* 93(29):e327)

Abbreviations: CID = combined immune deficiency, EOGT = EGF domain-specific O-GlcNAc transferase, HMIA = hereditary multiple intestinal atresia, MIA = multiple intestinal atresia, TEC = thymic epithelial cell, TTC7A = tetratricopeptide repeat domain–7A.

INTRODUCTION

Hereditary multiple intestinal atresia (HMIA), the rarest form of recurrent multiple atresia, was first reported by Winter and Zeltzer[1] in 1956. HMIA involves multiple atretic lesions along with homogenous intraluminal calcifications.[2] After the first association of HMIA and combined immune deficiency (CID) was reported,[3] multiple cases of HMIA associated with immune deficiency have been described.[4–9] The immune deficiency affects T- and B-cell functions, with lymphopenia, agammaglobulinemia, and impaired mitogen responses.[3–10] Death occurs before 2 years of age in most patients.

An autosomal recessive transmission for HMIA was postulated in 1973 by Guttman et al.[11,12] Through whole exome sequencing of 6 patients, we identified for the first time, 2 deleterious mutations in the gene tetratricopeptide repeat domain–7A (*TTC7A*), which are causative to the disease.[13] Additional publications have reported deleterious mutations in the *TTC7A* gene in HMIA patients in 15 unrelated families.[8–10] TTC7A mutations have also been identified in 5 infants with very early onset inflammatory bowel disease (VEOIBD).[14]

Human TTC7A protein contains 9 tetratricopeptide repeat domains, and its function has not been clearly established.[8,13] Proteins containing tetratricopeptide repeat domains are involved in numerous cellular processes, and therefore a plausible role of TTC7A in development might be suggested. Spontaneously arising mutations in the mouse *TTC7A* ortholog, *Ttc7* are known[15]: the flaky skin (*fsn*) mutation causes anemia, skin disorders (psoriasis), and gastric hyperplasia, and its thymus histology shows a markedly reduced cortex cellularity[16]; the *hea* mutation results in a lethal severe anemia, and the thymus in *hea* mice becomes atrophic early in life with an indistinct corticomedullar demarcation, few thymocytes, and a failure in the differentiation of $CD4^-$ $CD8^-$ double negative T cells to $CD4^+$ $CD8^+$ double positive cells.[17,18] Here, we evaluated the consequences of a defective TTC7A protein by analysis of

Editor: M José Carbonero Celis.
Received: October 16, 2014; revised: October 29, 2014; accepted: November 10, 2014.
From the Department of Microbiology, Infectiology and Immunology, University of Montreal, Montreal, Canada (IF, EH, FLD); Department of Microbiology and Immunology, CHU Sainte-Justine, Montreal, Canada (IF, FLD); CHU Sainte-Justine Research Center, Montreal, Canada (IF, NP, EH, HD, FLD); Department of Pathology and Cell Biology, CHU Sainte-Justine and University of Montreal, Montreal, Canada (NP); Department of Paediatrics, University of Montreal, Montreal, Canada (VM, EH, HD, FLD); Division of Gastroenterology, Hepatology and Nutrition, CHU Sainte-Justine, Montreal, Canada (VM); Histology Facility, IRIC, University of Montreal, Montreal, Montreal, Canada (MB); Medical Genetics Service, University of Sherbrooke, Sherbrooke, Canada (BM); and Division of Rheumatology, Immunology and Allergology, CHU Sainte-Justine Montreal, Canada (EH, HD, FLD).
Correspondence: Isabel Fernandez; Françoise Le Deist, CHU Sainte-Justine, Department of Microbiology and Immunology, 3175 Chemin de la Côte-Sainte-Catherine, Montreal, Quebec, Canada H3T 1C5 (e-mail: isabel.fernandez.hsj@ssss.gouv.qc.ca, francoise.le.deist@umontreal.ca).
The authors have no funding and conflicts of interest to disclose.

ISSN: 0025-7974
DOI: 10.1097/MD.0000000000000327

morphological patterns and TTC7A expression in several tissues from patients. Interestingly, we present new data on tissue abnormalities in patients and on the expression of TTC7A protein in the thymus at different stages of development (fetus and infant).

PATIENTS AND METHODS

Patients

Patients presenting multiple intestinal atresia (MIA) were identified at the Centre Hospitalier Universitaire Sainte-Justine in Montreal in the last 40 years. One affected 23-week-old fetus from Centre Hospitalier de l'Université de Laval in Quebec City was also included in this study (F). All patients were of French-Canadian origin, except P1 and P2 who had French-Canadian maternal origin and English partial paternal ancestry (Table 1).[13] This study was approved by the research ethics committees of CHU Sainte-Justine and CHU Laval.

P1 was the first child of nonconsanguineous parents without personal or familial history of congenital disorders. He was born at 33 weeks. Pregnancy was complicated by polyhydramnios. Repeat ultrasounds revealed intestinal distension and peritoneal calcifications. Type IV MIA and an atrophic microcolon were diagnosed. Multiple small bowel resections, jejunostomy, resection of most of the colon, and a Hartmann pouch construction were done. At 2 weeks of age, he underwent surgery for a pyloric web and 3 weeks later, stricturoplasties were performed on 3 new jejunal atretic segments. Because clinical signs of obstruction recurred, it was decided to desist from further surgical procedure, and the patient died at 47 days of life. The patient presented persistent lymphopenia (1.2 to 3.4×10^9/L for a normal range of 4.0 to 9.4×10^9/L) and monocytosis (1.7 to 5×10^9/L for a normal range of 0.2×10^9/L to 0.9×10^9/L).

P2, the second child of the same couple was born at 35 weeks of gestation with a prenatal diagnosis of intestinal obstruction and peritoneal calcifications. Surgery performed on the first day of life revealed MIA. At 4 weeks of age, the patient presented symptoms of intestinal obstruction and underwent resection of an atretic ileal segment. Because of the intestinal severe inflammation and persistent secretory diarrhea, an immunosuppressive treatment with corticosteroids and cyclosporine was initiated, but inflammation and diarrhea persisted. Liver biopsy at 6 months of age was consistent with parenteral nutrition associated liver disease. Before any treatment, the patient exhibited a severe immune deficiency characterized by profound agammaglobulinemia (IgA levels were <0.07 g/L, IgM <0.04 g/L, and IgG 0.33 g/L) and T-cell lymphopenia (3.0×10^9/L; normal range of 4.0 to 9.4×10^9/L), essentially characterized by a near absence of $CD8^+$ lymphocytes (0.12×10^9/L; normal range of 0.6 to 1.65×10^9/L) and monocytosis (1.5 to 7×10^9/L for a normal range of 0.2 to 0.9×10^9/L). He experienced 2 septic episodes. At 8 months, hematopoietic stem cell transplantation was performed using an HLA 6/6 matched cord blood. The intestinal disease progressed. Unfortunately, at 11 months of age, he developed respiratory distress and further progression of the intestinal stenosis, and the patient died shortly after.

P3 was a boy born in 1975 with a diagnosis of MIA, who died at 9 days of life.

P4 was a boy born in 1977, who died at 17 days of life. Clinical data reported MIA at the jejunum, cholestasis, hepatitis, peritonitis, and a surgery for an intestinal imperforation.

P5 was a boy who died at 17 days. He was born prematurely and presented MIA and a dilatation in the duodenum. He also exhibited hepatic steatosis and a probable cardiomyopathy.

P6 and F have already been described (see Table 1).[11–13]

P2 and F exhibited confirmed deleterious mutations in the *TTC7A* gene: a 4 base-pairs intronic deletion c.53344_53347delAAGT (F was homozygous and P2 was compound heterozygous for this mutation); a second missense mutation c.A133074G; p.L823P in P2. Note that P1 and P2 were brothers.[13] Unfortunately, TTC7A mutation was not established for the rest of the patients because the quality of the paraffin-embedded tissues did not allow adequate DNA extraction.

Histology and Immunohistochemistry

Samples from autopsies or biopsies (Table 1) were fixed in 10% buffered formalin, embedded in paraffin, and sections stained with hematoxylin and eosin using routine methods. All samples obtained from autopsy were analyzed; however, for practical reasons, we excluded the organs without morphological defects (such as kidney, pancreas, heart, muscle, and brain), and only clearly morphological abnormal organs were reported: intestine, thymus, spleen, liver, and lung. Tissues from age-matched children were used as control tissues.

Immunohistochemistry was performed on 3 μm paraffin-embedded sections, using an automated Ventana immunostainer (Benchmark XT Ventana Medical System Inc [VMSI], Tucson, AZ). We used the following commercial antibodies: CD20, CD68, CKN, CK7, and CK19 from DAKO (Glostrup, Denmark); CD3 and Ki67 from Cellmarque (Rocklin, CA); CD15

TABLE 1. Characteristics of HMIA Patients

Patients	Sex	Age of Death	Year	Previous Name	Reference	Recovered Tissues
P1*	M	47 d	2009	P14	[14]	Intestinal autopsy
P2*	M	11 mo	2010	P11	[14]	Intestinal biopsy Autopsy
P3	M	9 d	1975	NR		Autopsy
P4	M	17 d	1977	NR		Autopsy
P5	M	17 d	1971	NR		Autopsy
P6	M	17 d	1971	SS	[12,13]	Autopsy
F	F	23 wk fetus	1999	F4	[14]	Autopsy

HMIA = hereditary multiple intestinal atresia, NR = not reported.
* P1 and P2 were brothers.

(MMA) from Ventana (Tuckson, AZ); CD4 and CD8 from Vector (Burlingame, CA); β-catenin from BD Biosciences (Mississauga, Canada); TdT from Hot Springs, AR; γ-catenin from Abcam (Toronto, Canada).

TTC7A immunostaining on paraffin-embedded sections was carried out using the automated Discovery XT platform (VMSI). Sections were subjected to heat-mediated antigen retrieval with citrate buffer pH 6, then washed and subjected to blocking of nonspecific binding and endogenous peroxidase activity (VMSI). The TTC7A protein was labeled using a rabbit antihuman TTC7A antibody (ab122362, Abcam) that recognizes the sequence corresponding to amino acids 126–201 at the C-terminal of human TTC7A. The OMap anti-Rabbit Ig HRP (Multimer HRP) secondary antibody was applied at room temperature. Finally, slides were counterstained with hematoxylin and postcounterstained with sodium bicarbonate. Signal amplification was performed using the ChromoMap DAB detection kit. Sections were finally dehydrated and mounted, and then observed under a light microscope. Given that TTC7A is highly expressed in pancreas (http://www.proteinatlas.org), pancreas from control children were used as positive tissue.

RESULTS

Morphological Patterns of Intestinal Lesions and Immune Deficiency in Patients with HMIA

All cases studied (P1–P6 and F) presented the morphological intestinal anomalies characteristic of HMIA. An exhaustive immunohistochemistry study on the structural- and immune-features associated with HMIA was performed in bowel biopsies from P2 (Figure 1). Successive histological analysis revealed complete multifocal atresia with sieve-like multiple intestinal lumen caused by multiple mucosa adhesions. Micro and macro calcifications were observed randomly in the lumen. The epithelial crypts were dilated and contained mucus mixed with epithelial and inflammatory cells, particularly polymorphonuclear cells (PMN) and macrophages. Intestinal epithelium was partially or totally destroyed (Figure 1B and C) and, when preserved, appeared hypertrophic with packed cells. Moreover, epithelial cells were highly proliferative ($Ki67^+$ cells), even at the tip of villi (data not shown). At the same time, apoptosis of epithelial cells was observed. In preserved areas, 4/4 subtypes of intestinal epithelial cells (enterocytes, goblet cells, endocrine cells, and Paneth cells) were well represented, discarding a defect in epithelial cell differentiation. In destroyed areas, the epithelium was ulcerated and the mucosa was detached (the mucosa ''cry''), and enterocytes were present in the lumen and surrounded by fibers and inflammatory cells. At the epithelium, the β-catenin lost its usual basal–lateral localization (Figure 1D). A remarkable infiltration of $CD3^+$ T lymphocytes, mononuclear (data not shown), and largely PMN cells was noted in the lamina propria and lumen (Figure 1E). Scarce intraepithelial $CD8^+$ T lymphocytes were seen in epithelium and $CD4^+$ T lymphocytes in the lamina propria (data not shown).

Severe Alterations in the Thymus of Patients with HMIA

Postmortem examination of thymus performed in 5 patients (P2, P3, P4, P5, and P6) revealed alterations in organ architecture with severe atrophy, complete loss of corticomedullary demarcation, and major lymphoid depletion. However, in contrast to SCID patients, Hassall corpuscles were normally seen and thymocytes were present although scarcely. Immunostaining studies of thymus from 2 patients (P3 and P4)

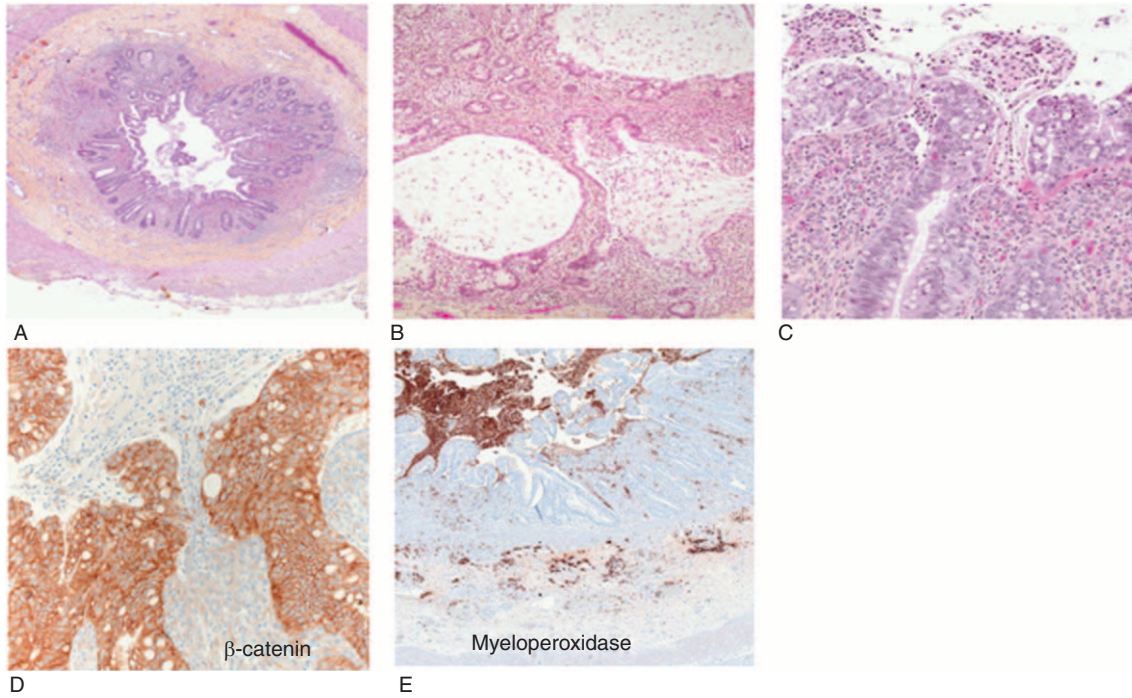

FIGURE 1. (A and B) Histological analysis of surgical samples from P2 revealed multifocal atresia. (C) The intestinal mucosa was severely injured. (D) The polarization of the epithelial cells was disturbed, as shown by the loss of β-catenin's basal–lateral localization. (E) A remarkable infiltration of myeloperoxidase PMN was noted. (A, B, and C: haematoxylin and eosin staining).

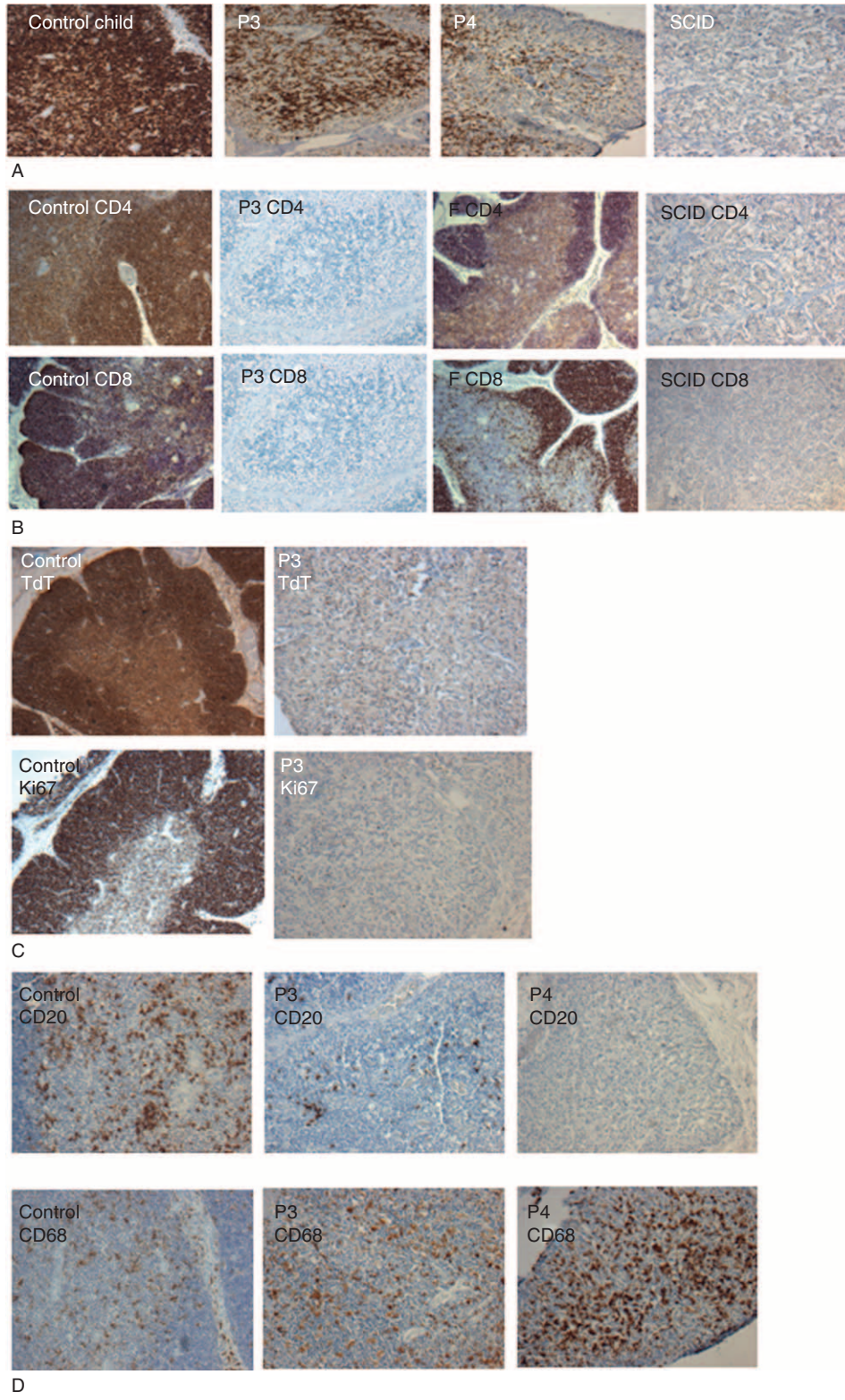

FIGURE 2. Thymus architecture appeared abnormal in HMIA patients. (A) Progressive loss of CD3 expression in thymocytes. (B) The thymus from HMIA children was empty of $CD4^+$ and $CD8^+$ lymphocytes. Thymus from a SCID patient with a missense mutation of the IL2RG gene is also shown. (C) Thymocytes from patients lost the ability to proliferate (TdT^- and $Ki67^-$ thymocytes). (D) Decrease in B-cell population ($CD20^+$) and (E) macrophage ($CD68^+$) accumulation was noted in HMIA thymus. HMIA = hereditary multiple intestinal atresia.

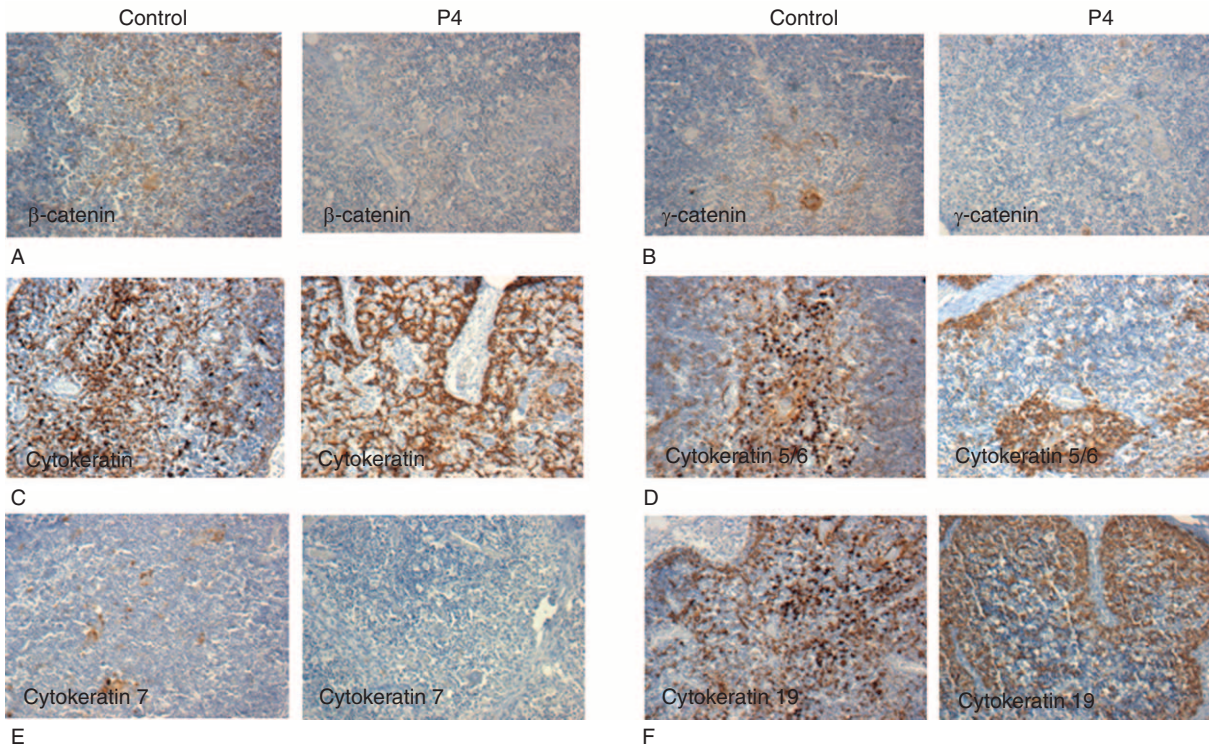

FIGURE 3. Drastic changes in expression and/or distribution of epithelial cell markers in HMIA thymus. (A) β-catenin, (B) γ-catenin, (C) cytokeratin, (D) cytokeratin 5 and 6, (E) cytokeratin 7, and (F) cytokeratin 19. HMIA = hereditary multiple intestinal atresia.

showed a marked reduction in $CD3^+$ cells and an absence of CD4 and CD8 expression in thymocytes (Figure 2A and B). $CD3^+$ lymphocytes were more numerous in the thymus of the younger patient P3 than in the older patient P4 (Figure 2A). The number of immature lymphocytes (TdT^+ cells) was clearly reduced and the thymocyte proliferation capability ($Ki67^+$ cells) impaired (Figure 2C). Moreover, B cells were rarely observed, whereas macrophages accumulated (Figure 2D and E). Intriguingly, in the 23 week-fetal HMIA thymus (F), a defined demarcation between cortex and medulla could be observed, although the cortex appeared markedly reduced. Furthermore, the presence of thymocytes seemed diminished as compared with the thymus of a 23-week-fetus and an infant. Both $CD4^+$ and $CD8^+$ thymocytes were seen in HMIA fetal thymus (Figure 2B).

The organization of the thymic stroma in patients revealed an absence of β-catenin at thymic epithelial cells (TECs) (Figure 3A) and partial or complete absence of γ-catenin at the Hassall corpuscle epithelium (Figure 3B). Moreover, the presence and distribution of cytokeratins in TECs seemed to be severely altered (Figure 3C–F), with a complete absence of cytokeratin 7, a cytokeratin typically located at the simple epithelium of Hassall corpuscles; expression in the whole stroma of total cytokeratins and cytokeratin 5 and 6, which are proteins normally restricted to medullar TECs; strong expression of cytokeratin 19 in cortex TECs, a protein that should be strongly expressed in medullar TECs.

HMIA is Associated with Multiorgan Failure

Abnormal thymus and intestinal lesions seen in our patients indicated that they presented HMIA associated with CID. Next, we investigated putative alterations in diverse tissues: spleen, liver, and lungs from 5 autopsies (P2, P3, P4, P5, and P6). The paucity of $CD4^+$ and $CD8^+$ T cell compartments was confirmed in the spleen, liver, and lung in P3 and P4 (Figure 4). In particular, spleen morphology appeared disorganized with the presence of some $CD3^+$ lymphocytes (more numerous in P3 than in P4) but absence of $CD4^+$ and $CD8^+$ T cells, together with macrophage and PMN cells infiltration (Figure 4A).

Histological alterations in the liver were observed in 4/5 patients (Figure 4B). Liver showed cholestasis, steatosis, and fibrosis, which could be consistent with liver disease associated with parenteral nutrition. However, it has to be considered that P3 (who died at 9 days) already had evidence of liver disease. For P3 and P4, severe macrophage and PMN infiltration and some $CD3^+$ lymphocytes, but neither $CD4^+$ nor $CD8^+$ T cells were noted (Figure 4B). Finally, lung autopsies demonstrated severe inflammation with some macrophage, but scarce $CD3^+$ lymphocytes (Figure 4C). Once again, neither $CD4^+$ nor $CD8^+$ lymphocytes were noted (Figure 4C). Overall, our results indicate that the HMIA disease is associated with morphological alterations in multiple organs besides the gastrointestinal tract.

Expression of TTC7A

Thymus, intestine, and pancreas from infants and HMIA patients were tested for TTC7A protein expression by immunohistochemistry. TTC7A protein was expressed in TECs and Hassall corpuscles in normal thymus (Figure 5). The same

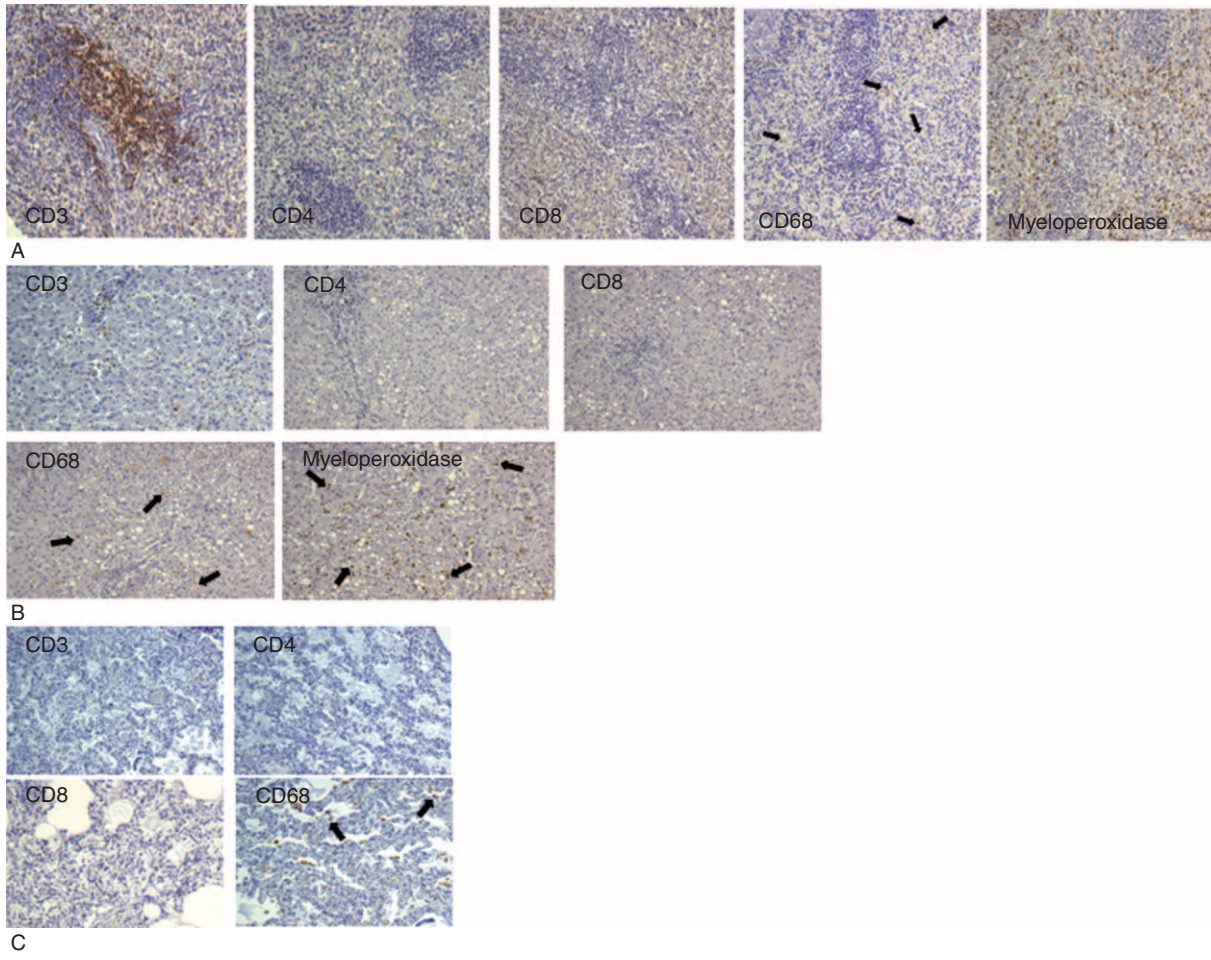

FIGURE 4. HMIA disease is associated with multiorgan alterations. (A) Presence of $CD3^+$ lymphocytes in the spleen of HMIA patients. Note the absence of $CD4^+$ and $CD8^+$ T cells and the presence of macrophages ($CD68^+$, arrow) and PMN cells (myeloperoxidase$^+$). (B) HMIA liver presented steatosis and $CD68^+$ macrophage (arrow) and myeloperoxidase$^+$ PMN cell infiltration (arrow). (C) HMIA lung appeared inflamed with $CD68^+$ macrophage infiltration (arrow). Note the presence of scarce $CD3^+$ T cells, the absence of $CD4^+$, and $CD8^+$ T cells in liver and lung. HMIA = hereditary multiple intestinal atresia.

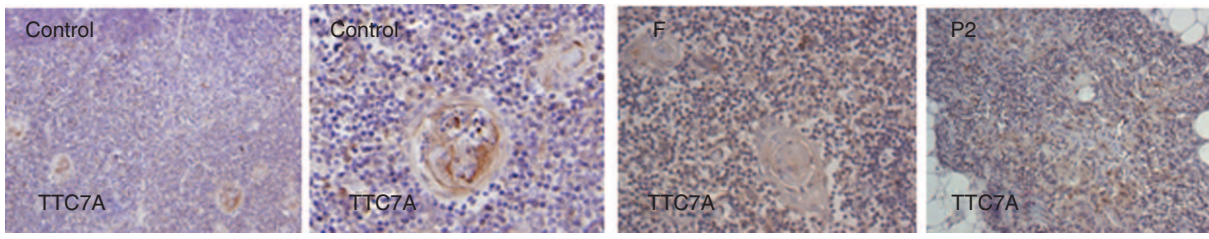

FIGURE 5. TTC7A was expressed in Hassall bodies and cells in the thymic stroma in healthy and HMIA thymus. HMIA = hereditary multiple intestinal atresia, TTC7A = tetratricopeptide repeat domain–7A.

TTC7A expression was noted in normal fetal thymus. In addition, in the stomach and intestine of control children (from newborn to 1 year old), TTC7A was expressed in epithelial, muscle, and endothelial cells (Figure 6A). Epithelial glandular cells from pancreas also expressed TTC7A protein, in both exocrine and endocrine epithelia (Figure 6B). Therefore, TTC7A protein seemed to be mainly present in cells from endodermic origin.

Surprisingly, TTC7A protein was highly expressed in epithelial cells from the intestine, thymus, and pancreas in samples from 4 patients tested (P1, P2, P3, and P4), and the fetus (F) (Figures 5 and 6). Although it could not be quantified,

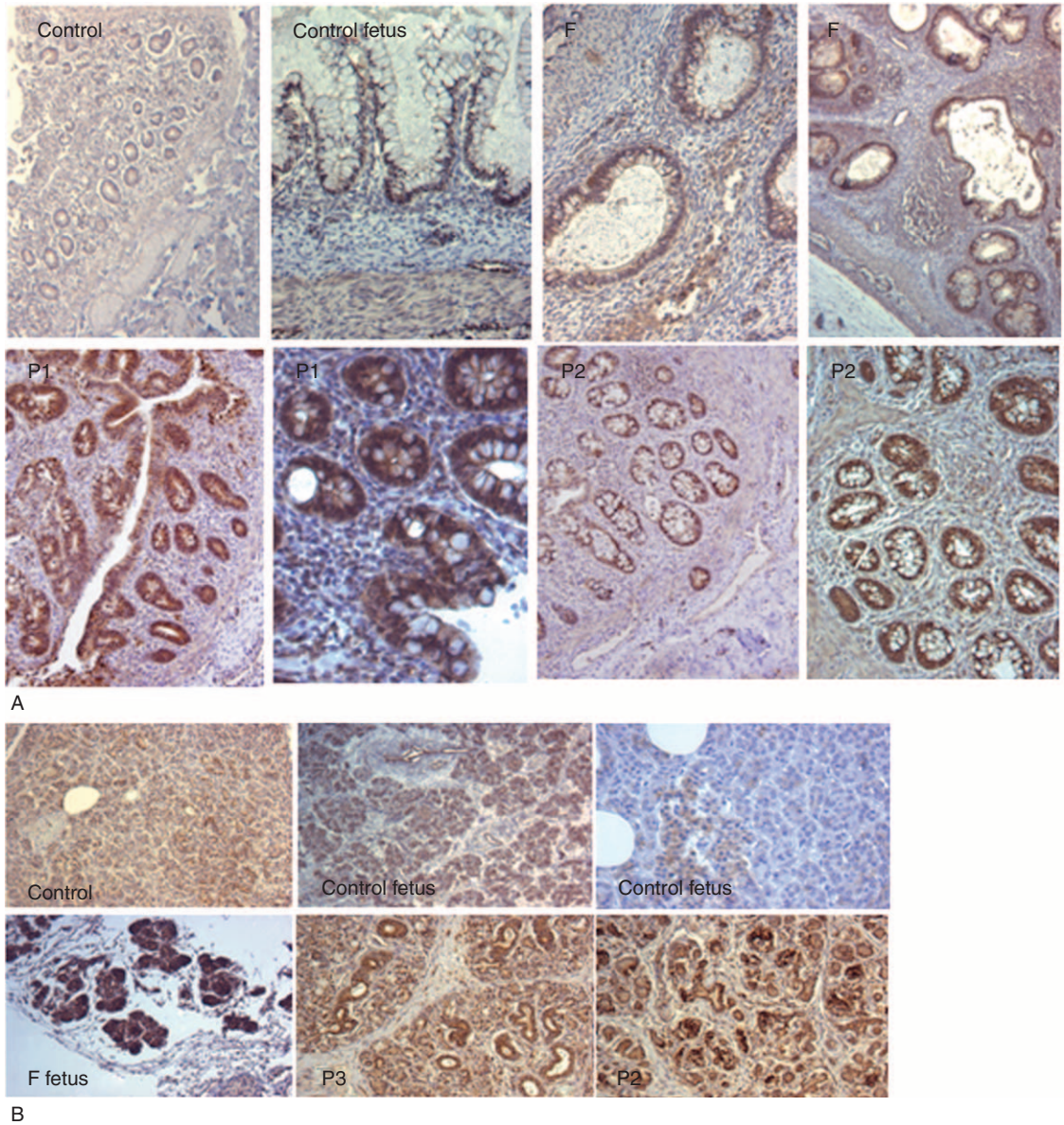

FIGURE 6. (A) TTC7A was expressed in intestinal epithelial cells and endothelial cells in controls and highly expressed in HMIA patients (F, P1, and P2). (B) TTC7A was expressed in normal epithelial cells from pancreas, in both exocrine and endocrine glands, and highly expressed in HMIA pancreas. HMIA = hereditary multiple intestinal atresia, TTC7A = tetratricopeptide repeat domain–7A.

mutated protein seemed to be more remarkable in the cell cytoplasm than the normal protein in control tissues. Therefore, in our patients, mutated *TTC7A* gene might likely encode a nonfunctional protein that accumulates inside the cells, mainly in epithelial cells.

DISCUSSION

In this study, we describe the alterations of different tissues, such as the intestine, thymus, lungs, spleen, and liver, which define HMIA-CID as multiorgan pathology. The thymic alterations together with the intestinal lesions support that all patients described here presented HMIA associated with CID. The reported ancestral TTC7A mutation has been considered as the unique hereditary cause of HMIA in French-Canadians,[13] a population that has been extensively studied for its founder effect and increased prevalence of hereditary diseases.[19] In this study of 7 Canadian HMIA cases, 5 were French-Canadian, and the homozygous ancestral mutation was reported in the fetus (F).[13] The others 2 patients were brothers and carriers of the ancestral mutation from the maternal side and another mutation from the paternal side.[13] Thus, we assume that our patients presented HMIA as a result of TTC7A mutation.

The architecture of the thymus in patients was profoundly altered, indicating a defect in epithelium development and/or maturation, which is probably the basis of the immune

deficiency. HMIA thymus exhibited low lymphocyte cellularity, deprivation of $CD4^+$ and $CD8^+$ T cells, an absence of β-catenin, a diminished expression of γ-catenin, and an abnormal distribution of all tested cytokeratins in TECs. The β- and γ-catenins are critical for cell-to-cell adhesion and then in the maintenance of tissue integrity. An absence of the typical catenin expression can be linked to abnormalities in thymus development and thymocyte differentiation because of the significance of these proteins in the Wnt-signaling pathway.[20–23] In each tissue from blood to gut, thymus, and spleen, we noticed a profound defect in T lymphocytes, mainly $CD8^+$ T lymphopenia. Very interestingly, the thymus architecture from the HMIA fetus appeared less affected, and CD4+ and CD8+ T thymocytes were present although diminished. Because F is homozygous for the French-Canadian ancestral TTC7A mutation, we assume that he will develop CID as the rest of the cohort. Consequently, the defect in tissue structure and T cell thymopoiesis seems progressive. Gradual loss of thymus function likely occurs because of the lack of crosstalk between maturing thymocytes and TECs. This crosstalk is crucial for the preservation of thymus architecture, TEC maturation, and thymus medulla formation.[24,25]

The expression of TTC7A protein in tissues of age-matched controls and patients was assessed. TTC7A protein was mainly expressed in TECs, intestinal, and pancreatic epithelial cells in infant and fetus controls. Unfortunately, the TTC7A antibody did not allow us to perform a double staining for double protein expression, and therefore, we were unable to report a clear presence of TTC7A protein in thymocytes. Intriguingly, defective TTC7A protein was highly expressed in all tissues from 4 patients and the fetus, which supports that the ancestral mutation results in the accumulation of the abnormal protein principally in the cytoplasm of the epithelial cells. The presence of the TTC7A protein in HMIA tissues is consistent with the described smaller size RNA observed from RNA of the fetus (F) versus RNA from control or HeLa cells.[13] We propose that a common defect caused by a mutation in the *TTC7A* gene during development of the thymus and the intestine (and may be other organs such as liver and/or lung), likely disrupting the epithelial integrity, is the main cause of HMIA associated with CID.

Recently, 2 reports have disclosed functional aspects of TTC7A protein in intestinal epithelial cells. However, a clear mechanism of TTC7A in CID and/or thymus abnormalities has not been proposed. In a very elegant study, Bigorgne et al[9] linked TTC7A deficiency with increased Rho kinase activity, which disrupted the integrity of the intestinal epithelial layer. However, a direct interaction of TTC7A with a particular component of the ρ-dependent pathway has not been revealed, and the increased activity of ρ kinase may be an indirect consequence of the TTC7A-dependent defective function at an undefined intracellular pathway/level. If TTC7A is involved in epithelial cell polarization, protein defects may strongly affect the highly polarized enterocyte, which may explain why intestine integrity is already affected at fetal stages. Although CID was present in almost all the patients, a potential role of the ρ kinase pathway in immune defects was not explored. RhoA has essential functions in T-cell development and is important for the survival and proliferation of T cell progenitors in the mouse thymus.[26] However, to our knowledge, a role for ρA in the development and/or integrity of the heterogeneous TEC population has not yet been described. In the second study, TTC7A mutation has been identified in 5 children with VEOIBD caused by defects in the PI4KA-TTC7A-EFR3B pathway. PI4KA is a critical regulator during *Drosophila* development, so we can assume that PI4KA might play a role in human development.[14] PI4KA protein expression is not reported in human epithelium of thymus and intestine (see expression patterns in Human Protein Atlas, http://www.proteinatlas.org). In this study, only 3/5 patients had CID characterized by lymphopenia, 2 of them with MIA, and the architecture of the thymus described in 1 patient seemed normal. Therefore, it is difficult to delimitate a clear link between PI4KA defect and CID in HMIA. A predicted functional partner of TTC7A (by STRING) is the EGF domain-specific O-GlcNAc transferase (EOGT) enzyme that is involved in development and is also a mediator of cell–cell and/or cell–matrix interactions at the cell surface.[27–29] Therefore, defective EOGT activity caused by TTC7A deficiency might perturb epithelial cell cytoskeleton and finally lead to a loss of epithelium integrity. Although we could speculate a role of TTC7A-EOGT in HMIA, neither bowel disturbances nor CID have been reported in EOGT deficiency.

Clinicians face a difficult situation as the severity of the intestinal presentation precludes long-term survival, whereas the importance of CID increases the risk of severe and/or opportunistic infections in these infants. This report reveals that a dysfunctional TTC7A protein explains a defective thymus. Therefore, isolated hematopoietic stem cell transplantation should be considered with caution in HMIA patients. Chen et al reported 1 patient who achieved an immune reconstitution 22 months after transplantation with mismatched sibling as donor.[8] However, the patient still had an atretic intestine and continued to be dependent on parenteral nutrition. On the contrary, after combined liver–small bowel transplantation for a child with HMIA,[30] liver and intestinal abnormalities did not relapse, and the reported allograft-derived lymphoid reconstitution had a phenotypic profile of only intraepithelial lymphocytes. Although little is known on the bone-marrow architecture and composition in HMIA patients, bone-marrow tissue from P4 was morphologically normal, and bone-marrow samples from 2 patients with TTC7A mutation showed a normal cellularity (data not shown). Therefore, a potential indication for bowel transplantation combined by thymus transplantation might be considered. Finally, how a dysfunctional TTC7A protein accumulates in cells and triggers mechanisms leading to HMIA-CID should be ascertained.

REFERENCES

1. Winter ST, Zeltzer M. Congenital atresia of the ileum in two brothers. *J Pediat.* 1956;4:194–196.
2. Lambrecht W, Kluth D. Hereditary multiple atresias of the gastrointestinal tract: report of a case and review of the literature. *J Pediatr Surg.* 1998;33:794–797.
3. Moreno LA, Gottrand F, Turck D, et al. Severe combined immunodeficiency syndrome associated with autosomal recessive familial multiple gastrointestinal atresias: study of a family. *Am J Med Genet.* 1990;37:143–146.
4. Rothenberg ME, White FV, Chilmonczyk B, et al. A syndrome involving immunodeficiency and multiple intestinal atresias. *Immunodeficiency.* 1995;5:171–178.
5. Shorter NA, Georges A, Perenyi A, et al. A proposed classification system for familial intestinal atresia and its relevance to the understanding of the etiology of jejunoileal atresia. *J Pediatr Surg.* 2006;41:1822–1825.
6. Conrad C, Freitas A, Clifton MS, et al. Hereditary multiple intestinal atresias: 2 new cases and review of the literature. *J Pediatr Surg.* 2010;45:E21–E24.
7. Ali YA, Rahman S, Bhat V, et al. Hereditary multiple intestinal atresia (HMIA) with severe combined immunodeficiency (SCID): a case report of two siblings and review of the literature on MIA,

HMIA and HMIA with immunodeficiency over the last 50 years. *BMJ Case Reports.* 2011doi:10.1136/bcr.05.2010.3031.

8. Chen R, Giliani S, Lanzi G, et al. Whole-exome sequencing identifies tetratricopeptide repeat domain 7A (TTC7A) mutations for combined immunodeficiency with intestinal atresias. *J Allergy Clin Immunol.* 2013;132:656–664.
9. Bigorgne AE, Farin HF, Lemoine R, et al. TTC7A mutations disrupt intestinal epithelial apicobasal polarity. *J Clin Invest.* 2014;124:328–337.
10. Agarwal NS, Northrop L, Anyane-Yeboa K, et al. Tetratricopeptide repeat domain 7A (TTC7A) mutation in a newborn with multiple intestinal atresia and combined immunodeficiency. *J Clin Immunol.* 2014;34:607–610.
11. Guttman FM, Braun P, Garance PH, et al. Multiple atresias and a new syndrome of hereditary multiple atresias involving the gastrointestinal tract from stomach to rectum. *J Ped Surg.* 1973;8:633–640.
12. Dallaire L, Perreault G. Hereditary multiple intestinal atresia. *Birth Defects Orig Artic Ser.* 1974;104:259–264.
13. Samuels ME, Majewski J, Alirezaie N, et al. Exome sequencing identifies mutations in the gene TTC7A in French-Canadian cases with hereditary multiple intestinal atresia. *J Med Genet.* 2013;50:324–329.
14. Avitzur Y, Guo C, Mastropaolo LA, et al. Mutations in tetratricopeptide repeat domain 7A result in a severe form of very early onset inflammatory bowel disease. *Gastroenterology.* 2014;146:1028–1039.
15. White RA, McNulty SG, Nsumu NN, et al. Positional cloning of the Ttc7 gene required for normal iron homeostasis and mutated in hea and fsn anemia mice. *Genomics.* 2005;85:330–337.
16. Beamer WG, Pelsue SC, Shultz LD, et al. The flaky skin (fsn) mutation in mice: map location and description of the anemia. *Blood.* 1995;86:3220–3226.
17. Shimizu K, Keino H, Ogasawara N, et al. Hereditary erythroblastic anaemia in the laboratory mouse. *Lab Anim.* 1983;17:198–202.
18. Kasahara Y, Shimizu K, Kuribayashi K. Developmental abnormalities of the thymus in hea/hea mutant mice. Developmental abnormalities of the thymus in hea/hea mutant mice. *Exp Anim.* 2008;57:85–94.
19. Laberge AM, Michaud J, Richter A, et al. Population history and its impact on medical genetics in Quebec. *Clin Genet.* 2005;68:287–301.
20. Staal FJ, Noort MM, Strous GJ, et al. Wnt signals are transmitted through N-terminally dephosphorylated beta-catenin. *EMBO Rep.* 2002;3:63–68.
21. Clevers H. Wnt/beta-catenin signaling in development and disease. *Cell.* 2006;127:469–480.
22. Xu M, Sharma A, Hossain MZ, et al. Sustained expression of pre-TCR induced beta-catenin in post-beta-selection thymocytes blocks T cell development. *J Immunol.* 2009;182:759–765.
23. Liang CC, You LR, Yen J, et al. Thymic epithelial b-catenin is required for adult thymic homeostasis and function. *Immunol Cell Biol.* 2013;91:511–523.
24. van Ewijk W, Hollander G, Terhorst C, et al. Stepwise development of thymic microenvironments in vivo is regulated by thymocyte subsets. *Development.* 2000;127:1583–1591.
25. Holländer GA, Wang B, Nichogiannopoulou A, et al. Developmental control point in induction of thymic cortex regulated by a subpopulation of prothymocytes. *Nature.* 1995;373:350–353.
26. Vielkind S, Gallagher-Gambarelli M, et al. Integrin regulation by RhoA in thymocytes. *J Immunol.* 2005;175:350–357.
27. Cohen I, Silberstein E, Perez Y, et al. Autosomal recessive Adams-Oliver syndrome caused by homozygous mutation in EOGT, encoding an EGF domain-specific O-GlcNAc transferase. *Eur J Hum Genet.* 2014;22:374–378.
28. Sakaidani Y, Nomura T, Matsuura A, et al. O-linked-N-acetylglucosamine on extracellular protein domains mediates epithelial cell–matrix interactions. *Nat Commun.* 2011;2:583.
29. Müller R, Jenny A, Stanley P. The EGF repeat-specific O-GlcNAc-transferase EOGT interacts with notch signaling and pyrimidine metabolism pathways in *Drosophila*. *PloS One.* 2013;8:e62835.
30. Gilroy RK, Coccia PF, Talmadge JE, et al. Donor immune reconstitution after liver-small bowel transplantation for multiple intestinal atresia with immunodeficiency. *Blood.* 2004;103:1171–1174.