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LETTER TO EDITOR

Novel Exonic Deletions in *TTC7A* in a Newborn with Multiple Intestinal Atresia and Combined Immunodeficiency

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To the Editor:

Hereditary multiple intestinal atresia and combined immunodeficiency (MIA-CID, also referred to as gastrointestinal defects and immunodeficiency syndrome (GIDID); OMIM: 243150; reviewed in Jardine and Muise 2019 [1]) is an extremely rare and typically lethal disorder. This condition is characterized by multiple intestinal obstructions with atretic sites occurring throughout the small and large intestines. In addition, the gastrointestinal manifestations can include very early onset inflammatory bowel disease (VEOIBD). These intestinal features are associated with a spectrum of immunodeficiency, ranging from mild lymphocytopenia to severe combined immunodeficiency. Total parental nutrition (TPN) dependence and recurrent bouts of sepsis caused largely by intestinal bacteria make the prognosis very poor [2], with very few children surviving beyond the first years of life. The genetic basis of this condition has recently been linked to autosomal recessive mutations in *TTC7A* [3–7]. In our patient, we demonstrate two novel deletions within *TTC7A* including a multi-exonic heterozygous deletion (exon 12–15) and a trans-allelic exon 15 deletion giving rise to MIA-CID complicated by very early onset inflammatory bowel disease and death in the first year of life. Novel interventions to treat patients with underlying *TTC7A* mutations are beginning to show promise including hematopoietic stem cell transplantation [8]; treatment for the dysregulation of the epithelium is yet to be well documented in these patients.

A male infant was born to non-consanguineous parents at 37 weeks gestational age. The mother was 34-year-old female with congenital absence of the left lower arm and hypothyroidism, who was otherwise healthy. The father was a healthy 37-year-old male. The couple's first child, a four-year-old female, was healthy. Prenatal ultrasound showed dilated, echogenic loops of bowel and a two-vessel umbilical cord; amniocentesis for genetic testing was declined.

At birth, the patient was non-dysmorphic with normal growth parameters. He had a mild systolic murmur, two-vessel umbilical cord, and a mildly distended abdomen. Abdominal radiographs showed no bowel gas distal to the stomach, suspicious for proximal bowel atresia. Contrast enema and upper GI study showed complete gastric outflow obstruction, normal rectum, and focal areas concerning small and large bowel atresia. Additional imaging revealed a moderate-sized apical muscular ventral septal defect, bilateral cystic renal dysplasia, an ectopic right kidney, multiple

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Table 1 Lymphocyte subsets and immunoglobulin levels in an infant with multiple intestinal atresia and combined immunodeficiency. ALC, absolute lymphocyte count

	Day30 of life	Age-specific reference values
ALC	1.21	$2.0–17.0 \times 10^9$/L
CD3	0.69	$2.30–7.00 \times 10^9$/L
CD4	0.61	$1.70–5.30 \times 10^9$/L
CD8	0.05	$0.40–1.70 \times 10^9$/L
CD19	0.4	$0.60–1.90 \times 10^9$/L
IgG	1.5	1.9–13.0 g/L
IgM	< 0.03	0.06–1.45 g/L
IgA	< 0.04	< 0.08 g/L

pancreatic cysts, a right hydrocele, and lenticulostriate vasculopathy.

On day two of life, the patient underwent exploratory laparotomy. The operative findings included a type 1 pyloric stenosis, type 1 multi-segment jejunoileal, and right colonic atresia. He underwent a Heineke-Mikulicz pyloroplasty with multi-segment jejunoileal resection requiring nine anastomoses and end ileostomy, with right hemicolectomy. Post-surgical bowel length was approximately 40 cm, reduced from the pre-operative bowel length estimated to be greater than 350 cm. Gross and microscopic examination (described later) suggested VEOIBD and resulted in further hematologic and immunologic workup. Laboratory examination at this time revealed low absolute lymphocyte count and significantly low T (both CD4 and CD8) and B cells. Immunoglobulins IgA and IgM were undetectable and IgG was low for age, although there is difficulty in interpreting at this age (Table 1). These values were compatible with a combined immunodeficiency phenotype. Protective isolation, no live vaccines, cytomegalovirus (CMV)-negative irradiated blood products, and monthly intravenous immunoglobulin dosed at 600 mg/kg were initiated, in addition to viral and bacterial prophylaxis.

At approximately 2 weeks of age, he developed intermittent blood per rectum, bridging his pathologic and clinical phenotypes which were concerning for VEOIBD. At 5 months of age, the patient remained on TPN with multiple unsuccessful attempts at enteric feeds. CMV viremia was first detected at 2 months of age, and despite targeted therapy, this infection was never well controlled with increasing viral copy numbers over time. Given the minimal therapeutic options, the family elected for palliative measures. In light of this decision, extended diagnostic workup (e.g., further definition of the immune phenotype and function) was not pursued. The patient died at the age of 10 months.

Gross examination of the atretic small bowel and colonic segments, removed at the time of surgery on day two of life, showed a uniform tapered appearance with no apparent interruption (Fig. 1a). Mesenteric defects were not identified at the

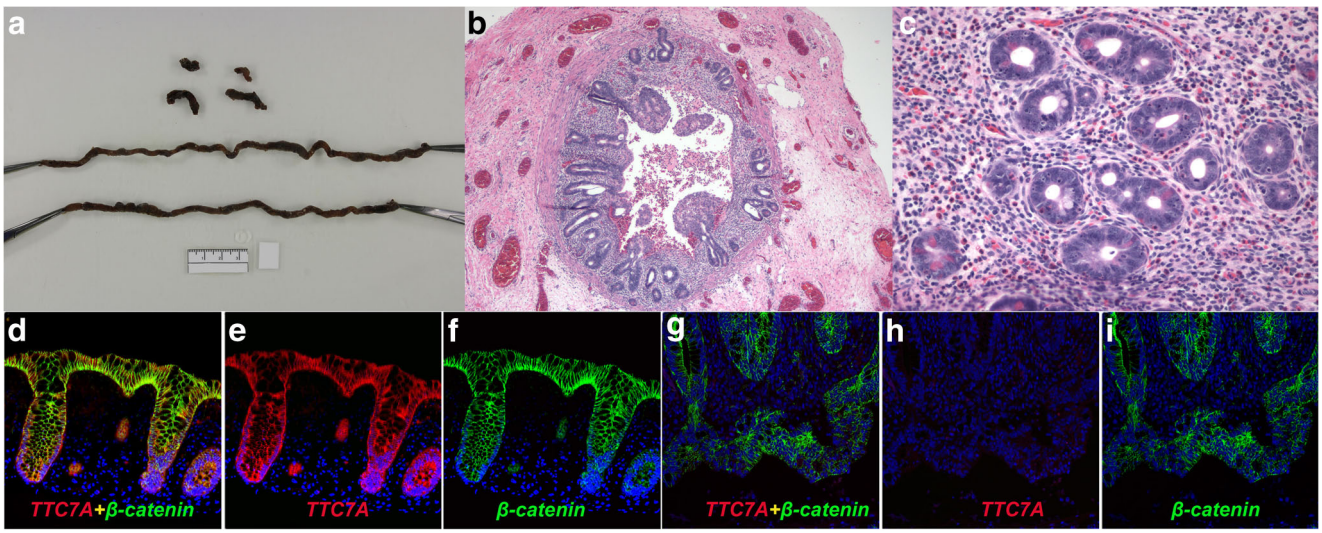

Fig. 1 Gross surgical specimen (**a**) showing multiple atretic bowel segments with complete fibrous obliteration of multiple areas. These atretic segments were found between otherwise normal appearing bowel segments. Photomicrographs of small bowel (**b**, **c**) of these atretic areas showing mucosal erosion, diffused apoptotic-like changes, and dense mixed inflammatory infiltrate within the lamina propria with marked eosinophilia (hematoxylin and eosin. **b**: ×40 original magnification, **c**: ×200 original magnification). Dual immunofluorescence labelling (**d**–**i**) of TTC7A and beta-catenin performed on formalin-fixed paraffin embedded tissue of gastrointestinal mucosa from control and the patient's tissue. The healthy control tissue shows overlap (**d**) staining of TTC7a (**e**) and beta-catenin (**f**). The patient's overlap panel (**g**) demonstrates complete absence of TTC7A (**h**) expression with retained beta-catenin expression (**i**), confirming TTC7A null expression status

time of surgery nor at gross examination. Routine microscopy showed a spectrum of changes (Fig. 1b, c) including narrowed to sieve-like bowel mucosa with multiple fibrotic lumina. In addition, patchy areas of complete fibrous obliterations of the mucosa with associated granulation tissue and prominent histiocytic and eosinophilic infiltrate within the lamina propria and submucosa were noted. In the non-obliterated regions, the surface epithelium showed erosion and diffuse apoptotic-like changes, particularly of note in the crypt bases.

A chromosomal SNP microarray (Cytoscan HD; Affymetrix) initially showed a deletion of ~13 Kb (chr2:47241344–47254577; hg19) thought likely affecting exons 12–14 of TTC7A. No additional chromosomal copy number variants were identified from the SNP array. Follow-up gene sequencing and targeted duplication/deletion testing using comparative genomic hybridization (Agilent 180 K) and a second SNP microarray (Illumina Infinium 850K v1.1 BeadChip) were performed. No single-nucleotide variants or small insertions or deletions in *TTC7A* were identified. The copy number variant (CNV) analysis, however, identified biallelic deletions in the proband affecting *TTC7A* (chromosome 2p21). The first deletion was between 11.6 and 12.8 kb in length (minimum g.47246165–47257759; maximum g.47245049–47257853; hg19) and corresponds to a deletion of intron 11–15 (exon 12–15). It was heterozygous in the father. The second identified deletion, heterozygous in the mother, was between 3.1 and 3.3 kb in length and corresponds to a deletion of intron 14 to intron 15 (exon 15). The patient was therefore compound heterozygous for two different deletions, which overlapped in exon 15, leading to homozygous deletion of exon 15 in the patient. These deletions resulted in null protein expression as evidenced by absent immunofluorescence staining of TTC7A (Fig. 1d–i). These large exonic overlap deletions are not reported in the Database of Genomic Variants consisting of curated sets of variants from a select number of high-resolution and high-quality studies.

We provide the first report of intragenic compound heterozygous deletions in *TTC7A* causing MIA-CID. In the past several years, around 50 occurrences of this disease have been reported. For our patient, prolonged TPN dependence, VEOIBD, and immunodeficiency were major causes of morbidity. We also report multiple malformations for the first time in this disease, namely, a large ventricular septal defect, ectopic right kidney, and bilateral cystic dysplasia of the kidneys. The mother's isolated unilateral transverse terminal upper limb defect was most likely a sporadic malformation unrelated to these other malformations. To rule out a second genetic disorder, the family was enrolled in a research study providing exome sequencing which yielded no additional pathogenic variants. The relationship of *TTC7A* deficiency to VEOIBD has been previously reported [4]; however, the role of *TTC7A* in ventral septal defect, ectopic kidney, and bilateral cystic kidneys has no precedent in the literature. This gene is broadly expressed and plays diverse roles in cellular functioning; it would not be surprising if there are additional phenotypes resulting from its loss that have yet to be recognized. For example, it has only recently been observed that an ichthyotic skin condition develops over time in a cohort of patients with *TTC7A* mutations [9].

Although the exact correlation between different genotypes and various phenotypes is unclear, the most severe phenotypes appear to be caused by nonsense mutations or frameshift insertions/deletions causing total abolition of protein function [7]. However, as reviewed by Kammermeier et al. [8], the specific type of mutation underlying the abolition does not appear to impact the clinical severity. The patient described here expresses a severe phenotype of MIA-CID with multiple large and small bowel atresia, combined immunodeficiency, and very early onset inflammatory bowel disease. In addition, numerous abnormalities which have not been previously described in the context of *TTC7A* gene mutation are seen. The null expression of the TTC7A protein within gastrointestinal enterocytes, as confirmed by immunofluorescence studies, suggests the total loss of TTC7A protein function, consistent with the severity of the phenotype.

Our report, likely the first to describe intragenic deletion affecting this gene, demonstrates the need to include copy number analysis when assaying for pathogenic *TTC7A* variants. Furthermore, if additional malformations are confirmed in other individuals with this disease, consideration should be given to routine screening renal ultrasound and echocardiography in newly diagnosed patients.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Jardine S, Dhingani N, Muise AM. TTC7A: steward of intestinal health. Cell Mol Gastroenterol Hepatol 2018:
2. Agarwal NS, Northrop L, Anyane-Yeboa K, Aggarwal VS, Nagy PL, Demirdag YY. Tetratricopeptide repeat domain 7A (TTC7A) mutation in a newborn with multiple intestinal atresia and combined immunodeficiency. J Clin Immunol. 2014;34(6):607–10.
3. 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(1):328–37.
4. 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(4):1028–39.

5. 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(3):656–664.e17.
6. 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(5):324–9.
7. Yang W, Lee PPW, Thong MK, Ramanujam TM, Shanmugam A, Koh MT, et al. Compound heterozygous mutations in TTC7A cause familial multiple intestinal atresias and severe combined immunodeficiency. Clin Genet. 2015;88:542–9.
8. Kammermeier J, Lucchini G, Pai SY, Worth A, Rampling D, Amrolia P, et al. Stem cell transplantation for tetratricopeptide repeat domain 7A deficiency: long-term follow up. Blood. 2016;128(9):1306–8.
9. Leclerc-Mercier S, Lemoine R, Bigorgne AE, Sepulveda F, Leveau C, Fischer A, et al. Ichthyosis as the dermatological phenotype associated with TTC7A mutations. Br J Dermatol. 2016;176:1061–4.

This case was awarded the Resident Recruitment Award and was presented as an abstract at the Society for Pediatric Pathology Fall Meeting (2018) by Dr. Jessica Saunders.