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LETTER TO EDITOR

Bialellic Mutations in Tetratricopeptide Repeat Domain 7A (*TTC7A*) Cause Common Variable Immunodeficiency-Like Phenotype with Enteropathy

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To the Editor:

Biallelic mutations in tetratricopeptide repeat domain 7A (*TTC7A*) gene have been shown to cause several overlapping clinical phenotypes. These include multiple intestinal atresia (MIA) with various degrees of combined immunodeficiency (CID) [1]; severe form of very early-onset inflammatory bowel disease, apoptotic enterocolitis (AE) [2]; and immune deficiency-related enteropathy-lymphocytopenia-alopecia (ELA) syndrome [3]. All affected individuals reported to date have presented in first few months of life and all suffered severe life-threatening gastrointestinal (GI) and/or immunological disease manifestations. In the case of MIA, surgical intervention is often necessary early in life. Disease progression and relapses of atresia and stenosis in many patients requires repeat surgeries and small bowel transplantation [4]. Many patients with enteropathy required total parenteral nutrition (TPN). Immunological studies of these patients showed varying degrees of hypogammaglobulinemia and lymphopenia which in some cases was consistent with a diagnosis of severe combined immunodeficiency (SCID).

Here, we report a case presenting with clinical features consistent with common variable immunodeficiency (CVID) and enteropathy but was later found to have compound heterozygous mutations in *TTC7A*. The patient presented at the age of 15 with lethargy, pallor, and a low body mass index. He was found to be anemic and had low levels of ferritin, folate,

Capsule Summary TTC7A deficiency typically causes severe gastrointestinal manifestations such as multiple intestinal atresia or early-onset inflammatory bowel disease. In some cases, this is associated with severe combined immunodeficiency. Partial loss-of-function mutations appear to be associated with a milder phenotype resulting in common variable immunodeficiency-like condition with enteropathy.

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calcium, and Vitamin D. He did not report any overt diarrhea, abdominal pain, or other symptoms suggestive of small bowel obstruction. He also had no prior history of recurrent or unusual infections. An esophago-gastroduodenoscopy showed an atrophic duodenal mucosa; there was no evidence of any atresia. Histology showed variable villous atrophy and marked lymphocytosis (Fig. 1c) with absence of plasma cells. He was found to have severe panhypogammaglobulinaemia with normal T and B cell numbers but almost absent class-switched memory B cells. Further investigations showed normal proliferative T cell response to PHA and anti-CD3 stimulation and normal neutrophil function tests (Table E1). The

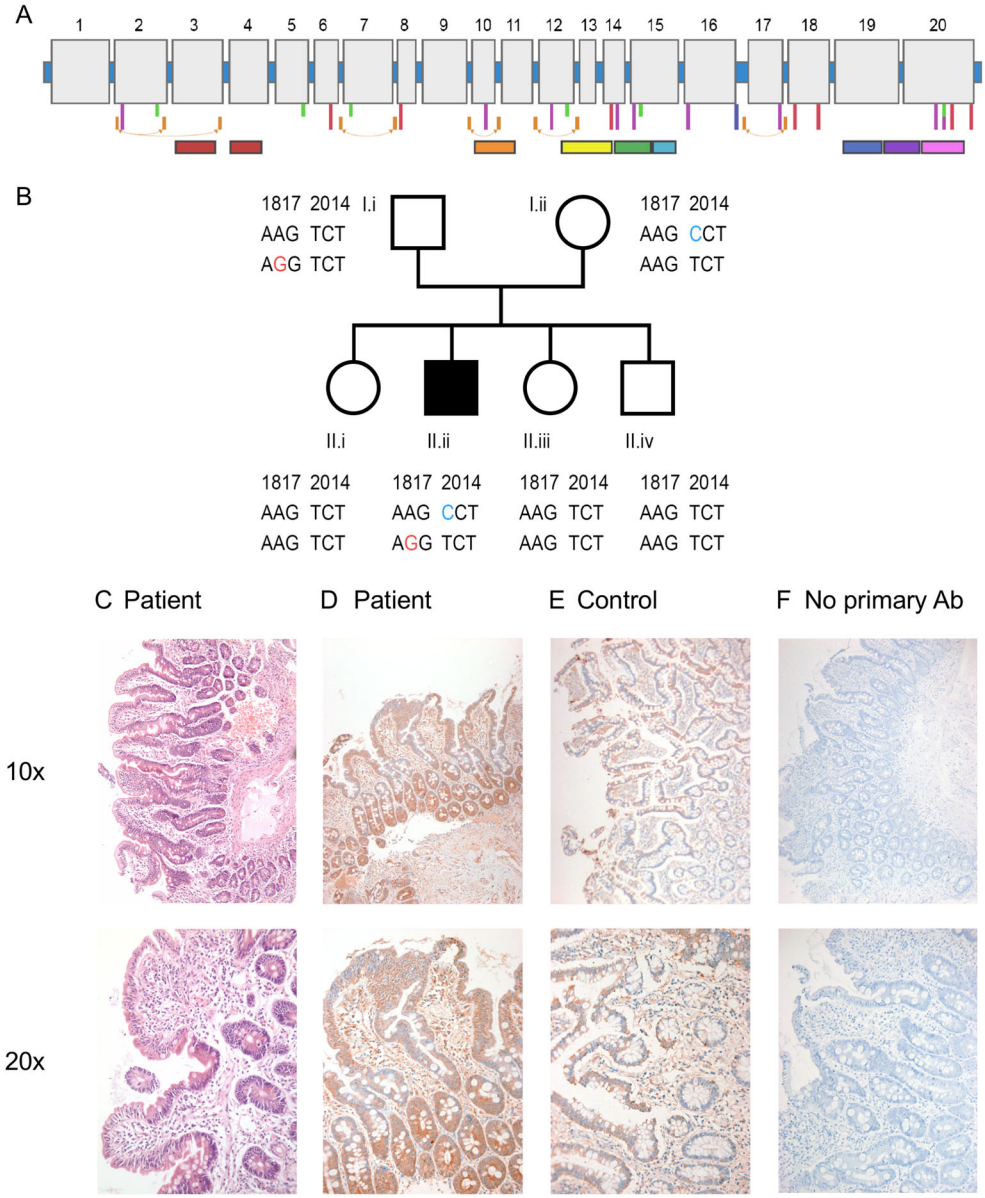

Fig. 1 Representation of TTC7A pathogenic variants with genetic and pathological findings. **a** Representation of the *TTC7A* gene and position of mutations identified to date in apoptotic enterocolitis, multiple intestinal atresias, combined immunodeficiency, and enteropathy-lymphocytopenia-alopecia. Vertical bars: protein changes, purple; stop codon, red; frame shift, green; exon skipping, curved arrows between orange bars; retained intron, blue. Horizontal bars: tetratricopeptide repeat domains (1–9) 121–157 and 177–210 red, 414–447 orange, 497–531 yellow, 533–565 green, 566–599 cyan, 745–778 blue, 780–812 purple, 813–846 magenta. **b** Pedigree and mutation inheritance pattern. **c** Gastrointestinal pathology. Duodenal biopsy with subtotal villous atrophy, marked crypt hyperplasia, and villous tip lymphocytosis. Goblet and Paneth cells are preserved. ×20 higher magnification—shortened villi with lymphocytosis and conspicuous lack of plasma cells within the lamina propria. **d** Immunohistochemistry of the patient's small intestine biopsy using rabbit polyclonal TTC7A antibody (Proteintech IL, USA). Hyperplasia is evident in comparison with healthy control **e**. No reduction in protein expression was seen. **f** Patient biopsy control with no primary antibody

Table 1 Genetic variants related to MIA-CID, AE, or ELA reported to date

Region	Nucleotide	Amino acid	Variant affect	Population	Phenotype	Reference	Family ID per ref.	Inheritance type
Exon 2	G214A	E71K	Missense	Caucasians/Sudanese	AE, CID, No atresia	Avitzur/Ngan*	1	CH
Exon 2	G214A	E71K	Missense	–	ELA	Lemoine	Fl	Homo/consanguineous
Skipping of 2–3	del Ex2c.185_348	del D62_ S116	Exon skipping	Mixed European	MIA-CID	Bigorgne	A	CH
Skipping of 2	del Ex2c.185_517	del D62_ G173	Exon skipping	Mixed European	MIA-CID	Bigorgne	A	CH
Exon 2	del Ex2c.313TATC	Y105fs	Frame shift	Serbian	MIA-CID	Chen	2	Homo
Exon 2	del Ex2c.313TATC	Y105fs	Frame shift	Bosniak	MIA-CID	Chen	3	Homo/related parents
Skipping of 2–3	Unknown mutations	–	Exon skipping	Italian	MIA-CID	Chen	8	Non-consanguineous
Exon 5	AG764A	K255fs	Frame shift	–	MIA-CID	Chen	4	CH
Exon 6	C833T	Q277X	Stop	Saudi Arabia	MIA-CID	Bigorgne	B	Homo/consanguineous
Skipping of 7	844-1G > T	–	Exon skipping	Caucasians	AE, MIA-CID	Avitzur	2	CH
Exon 7	delEx7c.911 T	L304fsX59	Frame shift	–	ELA	Lemoine	F2 P3	CH
Skipping of 7	del Ex7c.1000_1003AAGT	–	Exon skipping	French-Canadian and Mixed European	MIA-CID	Chen	5	CH
Skipping of 7	del Ex7c.1000_1003AAGT	–	Exon skipping	French-Canadian	MIA	Samuels	Fl F4 F6	Homo
Skipping of 7	del Ex7c.1000_1003AAGT	–	Exon skipping	French-Canadian/English	MIA-CID	Samuels	F7	CH
Skipping of 7	del Ex7c.1000_1003AAGT	–	Exon skipping	French-Canadian	MIA-CID	Fernandez	F	Homo
Skipping of 7	del Ex7c.1000_1003AAGT	–	Exon skipping	French-Canadian	MIA-CID	Fernandez	P2	CH
Exon 8	C1111G	Y337X	Stop	Mixed European	MIA-CID	Bigorgne	E	CH
Exon 9	T1198C	L400P	Missense	Italian	MIA-CID	Chen	7	Homo
Skipping of 10	Ex10c.1204-2A > G	–	Exon skipping	Caucasians	AE, MIA-CID	Avitzur	2	CH
Skipping of 12	Exon11–4 by deletion	–	Exon skipping	Norway	MIA-CID	Bigorgne	D	CH
Skipping of 12	Intronl2c.1510 + 105 T–A	del L465-A504	Exon skipping	Mixed European	MIA	Bigorgne	F	CH
Exon 12	1433 T > C	L478P	Missense	–	ELA	Lemoine	F2 P3	CH
Exon 12	del Ex12c.1479G	L493fsX13	Frame shift	Mixed European	MIA-CID	Bigorgne**	E	CH
Exon 14	C1576T	Q526X	Stop	Caucasians/Sudanese	AE, CID, No atresia	Avitzur/Ngan*	1	CH
Exon 14	C1616T	S539 L	Missense	Norway	MIA-CID	Bigorgne	D	CH
Exon 15	1652C > A	A551D	Missense	Irish/Ashkenazi Jew	MIA-CID	Agarwal	Fl	CH
Exon 15	Ins Ex15c.1673G	A558GfsX7	Frame shift	Mixed European	MIA	Bigorgne	F	CH
Ex 16 Read-through intron	del Ex16c.1919 + 1G > A	–	Retained intron	Arabic	MIA-CID	Chen	1	Homo/consanguineous
Exon 16	A1817G	K606R	Missense	French-Canadian and Mixed European	MIA-CID	Chen	5	CH
Exon 16	A1817G	K606R	Missense	European	CVID and enteropathy	Presented here	–	CH
Skipping of 17	1920-2A > G	–	Exon skipping	Malay	MIA-CID	Yang	Fld	CH

Table 1 (continued)

Region	Nucleotide	Amino acid	Variant affect	Population	Phenotype	Reference	Family ID per ref.	Inheritance type
Exon 17	T2014C	S672P	Missense	French-Canadian and Mixed European	MIA-CID	Chen	5	CH
Exon 17	T2014C	S672P	Missense	European	CVID and enteropathy	Presented here	–	CH
Exon 18	C2033A	S678X	Stop	Italian	MIA-CID	Chen	6	CH
Exon 18	C2134T	Q712X	Stop	Italian	MIA-CID	Chen	6	CH
Exon 20	T2468C	L823P	Missense	–	MIA-CID	Chen	4	CH
Exon 20	T2468C	L823P	Missense	French-Canadian	MIA-CID	Fernandez	P2	CH
Exon 20	T2468C	L823P	Missense	French-Canadian/English	MIA-CID	Samuels	F7	CH
Exon 20	C2482T	Q828X	Stop	Irish/Ashkenazi Jew	MIA-CID	Agarwal	Fl	CH
Exon 20	del 2496–2497 CG	A832fsX1	Frame shift	Sri Lanka	MIA-CID	Bigorgne**	C	Homo/likely consanguineous
Exon 20	G2494A	A832T	Missense	–	AE, No CID, No atresia	Avitzur	3	Homo
Exon 20	G2569 T	E857X	Stop	Malay	MIA-CID	Yang	Fld	CH

AE apoptotic enterocolitis, *MIA* multiple intestinal atresias, *CID* combined immunodeficiency, *ELA* enteropathy-lymphocytopenia-alopecia, *CH* compound heterozygous, *Homo* homozygous

[a] Ngan et al. independently report on the same case as Avitzur et al. with further work on WGS, thymus and lung pathology. Further features relating to phenotypes may be reported in indiviual references

[b] The dermatological phenotypes of patients C3 and E3 in Bigorgne et al. have also been recently reported [13]

features were consistent with diagnosis of CVID; coeliac disease could not be excluded. Gluten-free diet had only a modest effect at alleviating his symptoms. Although he had no history of infections, due to the severity of his panhypogammaglobulinaeamia, he was commenced on immunoglobulin replacement therapy. At the age of 17, the patient was diagnosed with type 1 diabetes mellitus following an admission for diabetic ketoacidosis. He remains free from infections but continues to have difficulties with malabsorption and poor weight gain.

The patient gave consent to participate in a study investigating molecular causes of primary immunodeficiencies. Two mutations in *TTC7A* were identified by whole exome sequencing (WES): rs139010200, exon 16/20 c.1817 aAg/aGg (K606R); and rs149602485, exon 17/20 c.2014 Tct/Cct (S672P). Sanger sequencing confirmed *TTC7A* variants in the proband as well as heterozygosity of A1817G paternally and T2014C maternally (Fig. 1b). No inheritance of affected alleles occurred in healthy siblings (Fig. E1) (for methods, please see online supplement).

These SNVs have been reported previously by Chen et al. (patient F5-A) on the maternal allele of European descent and in combination with exon 7 c.1000DAAGT on the paternal allele of French-Canadian descent [1]. Samuels et al. also report this same 4-nt deletion in a number of unrelated French-Canadian patients with MIA [5]. We suspect that compound heterozygous combination of K606R and S672P leads to a mild form of disease although we have not identified a clear mechanism by which this occurs. Confirmed pathogenicity of these variants will require in-depth functional analysis and reports of other similar instances.

The biological functions of TTC7A in MIA have been reviewed by L. Notarangelo [6] where this protein is suggested to act as a repressor of RhoA signaling. The administration of ROCK inhibitors is thought to ameliorate proliferative activity and epithelial architecture of the lumen and intestinal crypts. Avitzur et al. identify phosphatidylinositol 4-kinase IIIa (PI4KIIIa) as a major TTC7A-interacting protein [2]. The protein EFR3 homolog B (EFR3B) tethers TTC7A to the plasma membrane thereby allowing localization of PI4KIIIa at the cell membrane. In adult mice, the inactivation of murine PI4KIIIa leads to death due to necrosis of enterocytes in the villi and intestinal crypts [7]. However, the key immunological features of thymic dysplasia and lymphoid depletion in MIA-CID have not yet been explained.

The patient we presented here has a much milder clinical phenotype both in terms of his immunological and GI disease manifestations. We wondered if *TTC7A* variants found in this patient might have less detrimental effects on the function of the protein. To assess protein expression levels, immunohistochemistry was performed on the patient's small intestine biopsy using rabbit polyclonal TTC7A antibody (Proteintech IL, USA). Hyperplasia is evident in comparison with healthy control (Fig. 1d–f). No reduction in protein expression was seen. K606R is reported in EXAC with allele frequency 0.002061 including as homozygous. Given the mild phenotype presented here, it is not likely that this SNP alone produces a noticeable effect. A loss of fully functional protein may occur in combination with S672P without any visible reduction in expression. Genome, exome, and clinical panel sequencing in rare diseases generally requires filtering of common SNPs. The contribution toward disease due to mutations with allele frequency greater than 0.001 occurring in a biallelic state may be neglected in cases such as this.

Known variants associated with disease are shown in Table 1 and notated on the gene representation (Fig. 1a)[1–3, 5, 8–13]. This presents an updated list originally compiled by Yang et al. [8]. These variants were mapped on a model of the predicted TTC7A protein structure in Fig. E2. Modeling was based on the recently reported crystal structure of TTC7B [14] which has high sequence similarity to TTC7A. This reference data was combined with that of other tetratricopeptide repeat (TPR) domains found in a large numbers of proteins [6] (supplemental report E3 and E4). A confidence of 714 residues (81%) was modeled at > 90% accuracy. The TPR domains are identified as cartoons on the ribbon structure (Fig. E2).

The variants K606R and S672P have been described in patients as pathogenic but always in association with another more severe alteration. From our model, K606 and S672 lie buried on the beta-turn-beta between TPR domains 6/7 and may not be involved in major interactions resulting in a milder phenotype compared to other variants in the surrounding region.

Limited in vivo models exist for this condition, but it is hopeful that models of human intestine using pluripotent stem cells could allow greater functional study of *TTC7A*. A precise mechanism shared between gut development and robust immune function is yet to be identified. Our case expands the clinical phenotype associated with biallelic *TTC7A* mutations. Enteropathy is a relatively common complication of CVID, and as we continue to use more advanced genetic techniques to study this condition, it is possible that these less severe *TTC7A* variants will be found more frequently in this patient population.

Compliance with Ethical Standards The authors declare that they have no conflict of interest.

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