ACG CASE REPORTS JOURNAL

CASE REPORT | PEDIATRICS

Gastrointestinal Dysmotility Is a Significant Feature in 2 Siblings With a Novel Inositol 1,4,5-Triphosphate Receptor 1 (*ITPR1*) Missense Variant

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ABSTRACT

We present 2 siblings with a novel type 1 inositol 1,4,5-triphosphate receptor (*ITPR1*) missense variant who exhibit gastrointestinal dysmotility (chronic constipation and gastroparesis). *ITPR1* is expressed in the cerebellum and interstitial cells of Cajal. Periodic release of calcium by *ITPR1* initiates pacemaker currents, resulting in smooth muscle contraction. *ITPR1* mutations are known to be associated with neurologic syndromes, and these variants have not previously been associated with significant gastrointestinal manifestations in humans. Using whole-genome sequencing, *in silico* prediction software, biopsy samples, and manometry, the identified novel *ITPR1* variant is likely pathogenic and may have neurogastroenterology implications.

INTRODUCTION

Inositol 1,4,5-triphosphate (IP_3) receptor 1 (*ITPR1*) is an intracellular IP_3-gated channel that modulates calcium signaling through endoplasmic reticulum calcium release.[1] ITPR1 is found in neuronal and non-neuronal tissues, including the cerebellum, enteroendocrine cells, and interstitial cells of Cajal (ICC).[2,3] *ITPR1* gene variants have been associated with neurologic disorders such as spinocerebellar ataxia 15 (SCA15) (OMIM: 606658), congenital nonprogressive spinocerebellar ataxia 29 (SCA29) (OMIM: 117360), and Gillespie syndrome (OMIM: 206700).[4-6] However, mutations in *ITPR1* have not previously been reported in patients with gastrointestinal dysmotility. The siblings reported here suggest that, with further functional validation, a new likely pathogenic variant of *ITPR1* may represent a novel mutation that expands the phenotype of *ITPR1* syndromes already described or may expand the *ITPR1* disorders in the form of a new neurogastroenterology syndrome.

CASE REPORT

Patient 1: An Asian-American boy presented at 12 months of age with hypotonia and severe constipation. He required aggressive bowel regimens and underwent multiple hospitalizations for recurrent fecal impactions. He was diagnosed with gastroparesis with a liquid gastric emptying scintigraphy study (T 1/2: 630 min). Gastroduodenal and high-resolution anorectal manometry studies identified normal gastroduodenal and anorectal function with an intact rectoanal inhibitory reflex (Figure 1). Colonic manometry demonstrated lack of high-amplitude propagating contractions. He had extremely prolonged colonic sitz marker retention. Sibling 1's severe constipation is successfully managed with antegrade enema therapy through cecostomy tube.

ACG Case Rep J 2021;8:e00676. doi:10.14309/crj.0000000000000676. Published online: October 26, 2021

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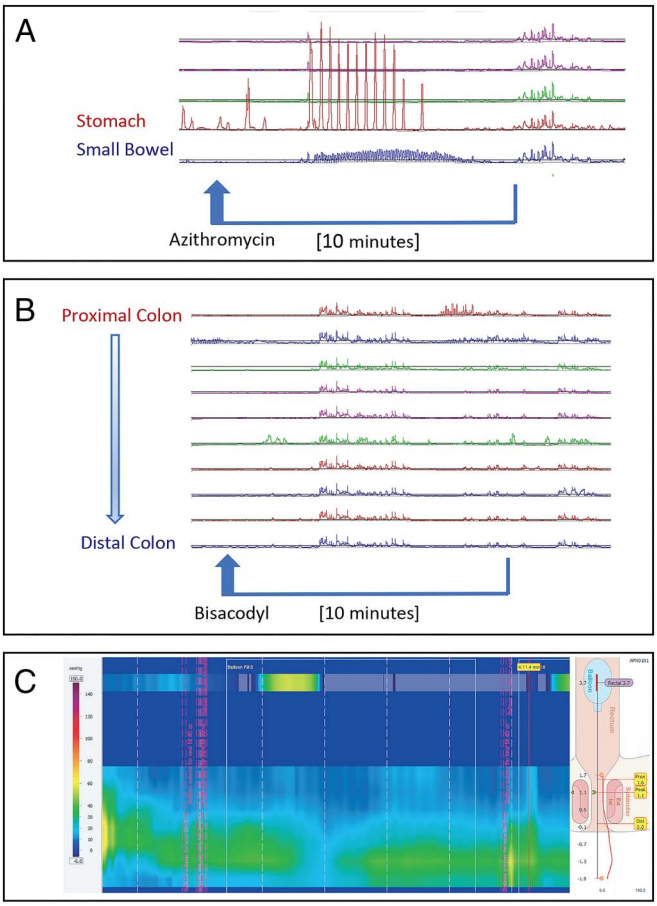

Figure 1. Gastroduodenal, colonic and anorectal manometry. (A) Gastroduodenal manometry in sibling 1 reveals intermittent early stomach contractions with subsequent small bowel contractions seen after azithromycin administration, indicating an intact motilin response. (B) Colonic manometry in sibling 1 identifies few colonic contractions with no high-amplitude propagating contractions identified, indicative of colonic inertia. (C) Anorectal manometry reveals intact rectoanal inhibitory reflex. In this study, the intrarectal balloon is inflated, resulting in the relaxation of the internal anal sphincter (IAS).

Additional diagnostic testing for sibling 1 included a normal muscle biopsy, chromosomal microarray, and global Metabolomic Assisted Pathway Screen. Histology from rectal suction biopsy identified normal ganglion cells, neurofibrils, and the presence of ICC (Figure 2).

From a neurological perspective, gross motor delays were identified at 6 months of age. Brain magnetic resonance imaging at 2 years identified asymmetric white matter changes in the left frontal lobe and mildly prominent ventricles and extra-axial spaces. At age 4 years, he developed absence and generalized seizures that responded to antiepileptic medications. Despite persistent symptoms of gastrointestinal dysmotility, a repeat neurologic evaluation performed at age 5 years demonstrated clinical improvements in speech and gross motor skills and a normal repeat brain magnetic resonance imaging.

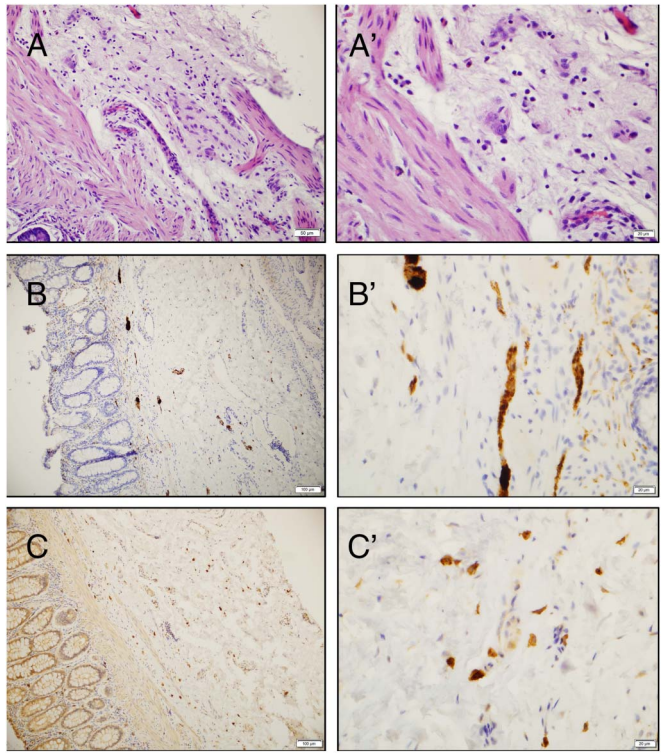

Figure 2. Sibling 1 endoscopic and rectal suction biopsy samples stained for ganglion cells, neurofibrils, and interstitial cells of Cajal. (A and A'): Ganglion cells appear normal with H&E stains at 20× and 40× magnification. (B and B'): Calretinin staining reveals normal ganglion cells with typical staining of lamina propria and neurofibrils appearance and number at 10× and 20× magnification. Vendor: Leica; clone: Cal6; titer: H2 (20); catalog number: PA0346 (C and C'): Interstitial cells of Cajal as stained by c-Kit (CD117) at 10× and 20× magnification with intermixed less intense staining of mast cells. Vendor: DAKO; rabbit polyclonal; titer: H1(20); catalog number: A450229.

Patient 2: A term girl presented with slow transit constipation, abdominal pain, and gastroparesis at 6 years of age. She requires 34 g of polyethylene glycol 3350 twice daily, a daily stimulant laxative, and intermittent rectal enemas for management of her constipation. She did not undergo manometry or sitz marker testing. As an infant, she was diagnosed with mild congenital hypotonia which resolved.

Whole-exome sequencing (WES) identified a heterozygous variant p.Ser1762Cys (AGT>TGT): c.5284A>T in exon 40 of the *ITPR1* gene (NM_002,222.5) in both siblings, but not in the biologic mother and was confirmed with Sanger sequencing (Figure 3A). Paternal genetic testing was unavailable (sperm bank donation, same donor). No other potentially deleterious WES polymorphisms or mutations associated with mitochondrial metabolism were identified. The identified variant is in the ITPR1 coupling/regulatory domain(Figure 3B), and the resulting amino acid substitution occurs in a position conserved across several species, including rats and humans. Numerous in silico predictions (PolyPhen-2,[7] Mutation t@ster,[8] Provean,[9] and Align GVGD[10]) indicated that the variant is deleterious (significant

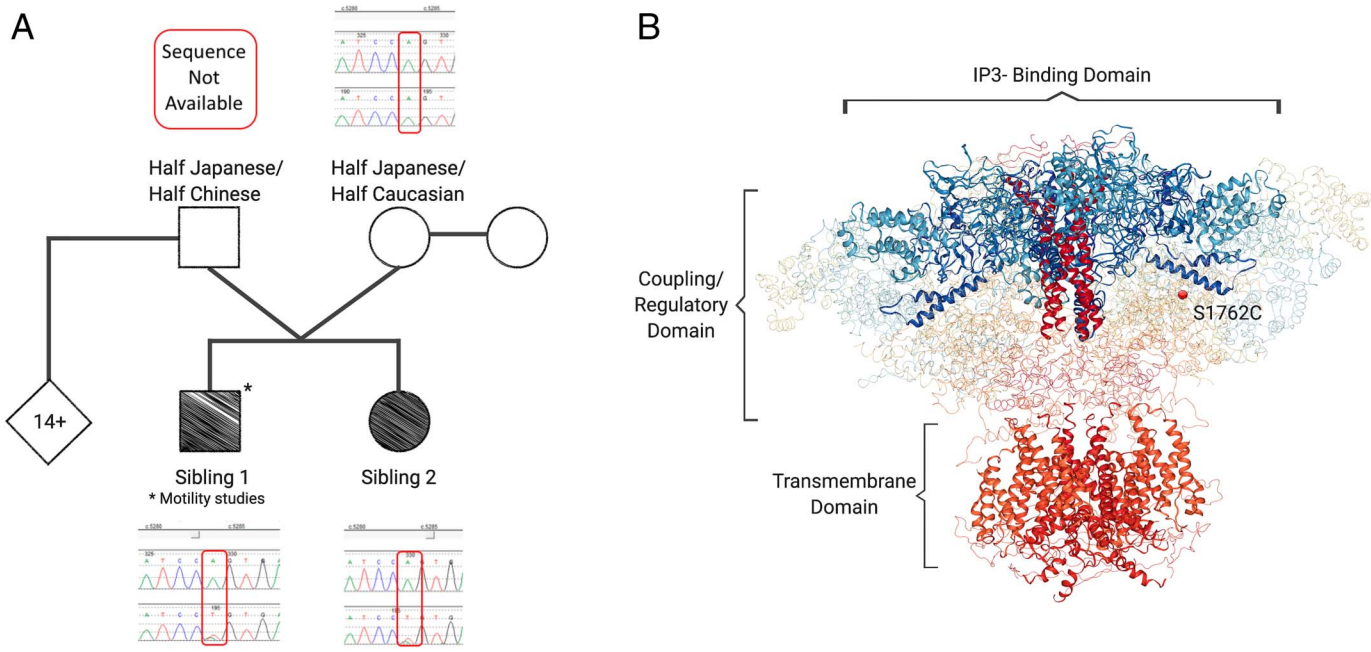

Figure 3. Confirmatory Sanger sequencing of mutation and mapping on ITPR1 protein structure in patient and sibling. (A) Sanger sequencing was used to confirm the c5284A> T mutation in exon 40 of ITPR1. Siblings 1 and 2, but not biological mother, have the mutation. Biological father's (sperm donor) mutation status unknown. (B) Mutation mapped to exposed region next to helix on ITPR1 protein in the coupling/ regulatory domain[20].

effect on protein function). The frequency for this mutation in the 1000 Genomes Project is 0.0002 (1/5008).[11]

DISCUSSION

We describe for the first time 2 siblings with a novel *ITPR1* variant in which neurogastrointestinal manifestations are a significant clinical feature. Heterozygous mutations in *ITPR1* have been implicated in 3 distinct human disorders, all of which are ataxia syndromes: SCA15, SCA29, and Gillespie syndrome. In humans, many gastrointestinal diagnoses occur before, or concomitantly with, the diagnosis of neurological disorders,[12] likely because of the similarities in neuroanatomy and neurochemistry of the brain and enteric nervous system where common pathogenic mechanisms may lead to both central nervous system and gastrointestinal dysfunction.[13] Both siblings (particularly sibling 1) had clinical features and characteristics present in other syndromes associated with heterozygous *ITPR1* mutations such as epilepsy, delayed motor development, hypotonia, and mild intellectual disability. Although the siblings are not ataxic, it is possible that the neurologic symptoms identified in sibling 1 may be part of a spectrum of neurologic disorders associated with this gene mutation. Variable expression is seen with other genetic neurologic disorders including those associated with SCN8a genetic mutations.

ICC currents generate electrical slow waves, facilitating phasic contractions, and are initiated by mitochondrial uptake of periodic release of calcium by *IItpr1*.[14,15] ICC abnormalities may lead to gastrointestinal motility disorders such as slow transit constipation and gastroparesis.[14,16] Previous work in *Itpr1* knockout mutant mice indicated that ITPR1 affects gastric slow-wave generation. Although these mice had normal ICC and enteric neuronal development, they lacked gastric slow waves, suggesting that gastric slow waves require ICC calcium release.[17] Consistent with the animal model, histopathology in sibling 1 identified typical c-Kit expression (indicating normal presence of ICCs) and normal enteric nervous system histology (Figure 2). The siblings' presentations of constipation parallel the phenotype of a conditional *ITPR1* knockout model with impaired colonic motility (delayed transit time, abdominal distension, and reduced colonic spontaneous contractions).[18] These animal model findings related to *ITPR1* increase the likelihood that the identified mutation in the siblings is clinically relevant to gastroenterology.

It should be noted that our findings meet Academy of Medical Genetics and Genomics and the Association for Molecular Physiology joint consensus guidelines,[19] suggesting that the amino acid change resulting from the identified *ITPR1* variant is "likely pathogenic." The lack of clinical and sequencing information from the biological father leaves unanswered questions regarding the nature of the variant such as if the variant occurred de novo, if there is variable penetrance or expressivity, if there is gonadal mosaicism, or if genetic anticipation may be involved. In addition, this report illustrates that WES may provide a tool to discover and diagnose genetic disorders affecting both the brain and gut enteric nervous system.[20]

In summary, we report 2 siblings with novel *ITPR1* variants with gastrointestinal dysmotility as a significant clinical feature. Although the variant is likely pathogenic, further investigation is required to fully elucidate the physiologic implications of the identified *ITPR1* variant.

DISCLOSURE

Author contributions: N.E.B. Tjaden wrote the manuscript and is the article guarantor. E. Chiou and B.P. Chumpitazi edited the manuscript. D.A. Schady provided the histopathology images. N.R. Pillai helped with genetic analysis. All authors critically read and approved the final manuscript.

Acknowledgments: We recognize the support of the Baylor College of Medicine Pediatric Residency Program, Mark Ward and Elaine Fielder, and the Pediatrician Scientist Training and Development Program (PSTDP), including Audrea Burns, William Parsons, Carl Allen, and Patricia Perez for their generous intellectual support.

Financial disclosure: This study was supported by the Texas Medical Center Digestive Disease Center (P30 DK 056338). N.E. Butler Tjaden was supported by the Baylor College of Medicine Pediatrician Scientist Training and Development Program. There are no competing interests.

Previous presentation: This case was presented at the Federation of Neurogastroenterology and Motility, September, 2018; Amsterdam, the Netherlands.

Informed consent was obtained for this case report.

Received March 2, 2021; Accepted June 21, 2021

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