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Expanding the genotypic spectrum of ACTG2-related visceral myopathy

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Abstract Visceral myopathies (VMs) encompass a spectrum of disorders characterized by chronic disruption of gastrointestinal function, with or without urinary system involvement. Pathogenic missense variation in smooth muscle γ -actin gene (*ACTG2*) is associated with autosomal dominant VM. Whole-genome sequencing of an infant presenting with chronic intestinal pseudo-obstruction revealed a homozygous 187 bp (c.589_613 + 163del188) deletion spanning the exon 6–intron 6 boundary within *ACTG2*. The patient's clinical course was marked by prolonged hospitalizations, multiple surgeries, and intermittent total parenteral nutrition dependence. This case supports the emerging understanding of allelic heterogeneity in *ACTG2*-related VM, in which both biallelic and monoallelic variants in *ACTG2* are associated with gastrointestinal dysfunction of similar severity and overlapped clinical presentation. Moreover, it illustrates the clinical utility of rapid whole-genome sequencing, which can comprehensively and precisely detect different types of genomic variants including small deletions, leading to guidance of clinical care decisions.

[Supplemental material is available for this article.]

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Ontology terms: chronic constipation; hypoperistalsis; ileus; intestinal pseudo-obstruction

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INTRODUCTION

Visceral myopathies (VMs), or congenital defects of the smooth muscle, can lead to several clinically overlapping disorders of the gastrointestinal (GI) system, including chronic intestinal pseudo-obstruction (CIPO) and megacystis microcolon intestinal hypoperistalsis syndrome (MMIHS). The main features of these disorders are abdominal pain and distension, constipation, and emesis. In MMIHS, bladder distension may also be present. An estimated 100 new CIPO cases are diagnosed each year in the United States, whereas a total of 227 MMIHS cases were reported in the literature between 1976 and 2011 (De Giorgio et al. 2011; Gosemann and Puri 2011).

Enteric smooth muscle γ -actin 2 (*ACTG2*) encodes an actin isoform that is selectively expressed in the smooth muscle layers of the GI tract (including the muscularis mucosa and muscularis propria) and is absent from skeletal muscle, heart, lung, and brain (Miwa et al. 1991; Halim et al. 2016). The muscularis propria is composed of two smooth muscle layers, the outer longitudinal layer and inner circular layer, which are innervated by the enteric nervous system and contract in an alternating pattern termed peristalsis to propel the contents GI tract onward (Lehtonen et al. 2012). Like other actin isoforms, *ACTG2* forms multimers within muscle cells to control cellular contractility in conjunction with myosin. Pathogenic

variation in *ACTG2* disrupts this crucial function and has been estimated to account for ~60% of VM cases (Assia Batzir et al. 2019). Several other genes have been implicated in VM, including myosin subunits *MYH11* and *MYL9*, and proteins involved in the actin–myosin interaction such as *LMOD1* and *MYLK* (Ambartsumyan 1993; Ravenscroft et al. 2018; Kandler et al. 2020). Enteric neuropathies such as Hirschsprung disease can also lead to CIPO (Ambartsumyan 1993). The disease progression for *ACTG2*-related visceral myopathy appears to be more severe than that of *ACTG2*-negative visceral myopathy, with high rates of parenteral nutrition dependence, visceral transplantation, and disease-related mortality (Assia Batzir et al. 2019).

In more than 50 individuals reported in the literature, heterozygous missense variants in *ACTG2* have been associated with autosomal dominant (AD) visceral myopathy, manifesting variably as CIPO or MMIHS (Lehtonen et al. 2012; Holla et al. 2014; Thorson et al. 2014; Wangler et al. 2014; Klar et al. 2015; Tuzovic et al. 2015; Lu et al. 2016; Milunsky et al. 2017; Ravenscroft et al. 2018; Assia Batzir et al. 2019). Functional studies have demonstrated that the *ACTG2* variants carried by these individuals lead to defects in actin polymerization and cellular contractility (Lehtonen et al. 2012; Thorson et al. 2014; Halim et al. 2016). Two cases of apparent autosomal recessive (AR) *ACTG2*-associated visceral myopathy (*ACTG2*-VM) have also been reported—one involving a homozygous loss-of-function (LOF) variant and the other involving a homozygous missense variant (Monies et al. 2017; Matera et al. 2021).

Here we present the case of a young child with severe chronic intestinal pseudo-obstruction, constipation, and bilious emesis. Whole-genome sequencing revealed a 187-bp homozygous deletion within *ACTG2* that is predicted to result in a complete loss of functional γ -actin protein. A family history negative for severe GI abnormalities suggests that *ACTG2* may display allelic heterogeneity, with disease caused by either monoallelic missense variation or biallelic LOF or missense variation.

RESULTS

Clinical Presentation and Family History

The patient, a former full-term infant born in Mexico, was reportedly healthy at birth but began outpatient treatment for constipation at 3 mo of age and presented with worsening constipation, abdominal distension, and bilious emesis at 8 mo of age. During the next 5 mo, the patient was hospitalized multiple times in Mexico and underwent multiple operations, including an ileostomy and lysis of adhesions. His symptoms failed to improve, and he was brought to San Diego, California for further evaluation.

Upon admission to Rady Children’s Hospital, the patient was 13 mo of age and weighed 6.8 kg (<1%ile, –3.6 SD, per Centers for Disease Control [CDC] U.S. growth charts). An abdominal radiograph revealed severely dilated bowel loops throughout the abdomen (Fig. 1A). Initial treatment included rehydration and nutritional optimization via nasogastric (NG) tube and peripherally inserted central catheter (PICC) line. He was later advanced to regular feeds with total parenteral nutrition (TPN) titrated to help the patient meet nutritional goals. Small bowel follow-through imaging showed normal transit time at 75 min, and retrograde contrast enema via rectum did not display any obstruction. Biopsies of the rectum and small intestine (ileostomy site) revealed abundant ganglion cells in both tissues, ruling out Hirschsprung disease. The muscular layers of the ileostomy site were unremarkable in thickness and organization (Fig. 1B).

Based on these findings, the decision was made to proceed with ileostomy takedown. Postoperative recovery was complicated by worsening abdominal distension, fevers, and sepsis-like symptoms. A computed tomograph (CT) of the abdomen demonstrated dilated

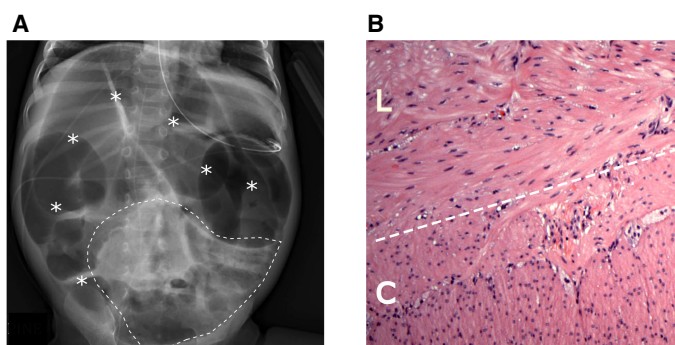


Figure 1. (A) Abdominal radiograph shows severe diffuse gaseous distension of bowel throughout the abdomen (asterisks). Scattered bubbly lucencies (within dashed line) may represent stool contents. Enteric tube and peripherally inserted central catheter (PICC) tip are also visible. (B) Hematoxylin and eosin (H&E) staining of the patient's small bowel (ileostomy) specimen reveals no abnormalities in smooth muscle band thickness or organization. (L) Longitudinal layer, (C) circular layer.

loops of bowel with a possible closed loop obstruction. The patient underwent an urgent exploratory laparotomy on postoperative day 7, during which viable bowel was visualized and adhesions were lysed.

Genomic Analyses

After 6 wk of hospitalization and in the absence of a clear diagnosis, the patient was nominated for clinical rapid whole-genome sequencing. A family history provided by the patient's mother at the time of genome sequencing revealed a history of recent intermittent constipation in a maternal half-sister (II-5) and fourth-degree consanguinity of the parents (Fig. 2A). Whole-genome sequencing of blood-derived DNA from the patient and his mother showed that the patient carried 56 regions of homozygosity (ROH) larger than 1 Mb, totaling 129.1 Mb (Supplemental Table 1). A sample from the father was not available. This total ROH is consistent with the distant parental consanguinity noted in the family history (coefficient of inbreeding $F \leq 1/32$) (Fan et al. 2013).

Within a 4.8-Mb region of homozygosity on Chromosome 2, a homozygous 187-bp intragenic deletion within the *ACTG2* gene was detected in the patient (Chr 2:74140750-74140936 × 0) (Tables 1 and 2). This variant was detected by one of the two copy-number variant (CNV) callers used in the genomic analysis pipeline: Manta but not CNVnator, consistent with Manta's higher sensitivity for CNVs 100 bp to 1 kb (Kosugi et al. 2019). It was not detected through DRAGEN-based single-nucleotide variant (SNV)/indel calling. The deletion spanned 24 bp of the 3' end of exon 6 (out of 9) of *ACTG2*, and the following 163 bp of intron 6, denoted as c.589_613 + 163del188 (NM_001615.3) (Fig. 2B–D). The deletion was present in the heterozygous state in the mother and was presumed heterozygous in the father, who was not available for testing, given the homozygous status in proband and known parental consanguinity (Fig. 2C; Supplemental Table 1). Multiplex ligation-dependent probe amplification was used to orthogonally confirm these results in the patient's and mother's samples. This deletion is absent from population databases gnomAD SV and DGV. The deletion creates a frameshift ending in a premature stop codon at position 80, which is predicted to lead to nonsense-mediated decay of aberrant *ACTG2* transcripts from both alleles in the patient.

In previous studies, intestinal biopsies from patients with AD *ACTG2*-VM have shown thinning of the longitudinal muscle layer and a disordered arrangement of smooth muscle fibers in the circular and longitudinal muscle bands of the muscularis propria (Tuzovic

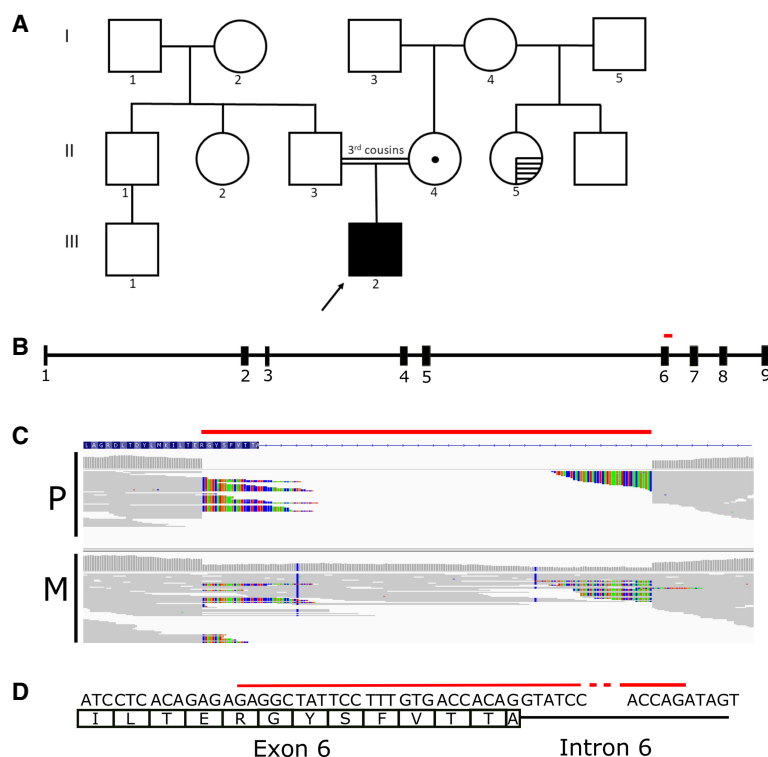


Figure 2. (A) Pedigree of the patient (III-2), indicated with the arrow. The dot (II-4) indicates heterozygous carrier status; horizontal hatching (II-5) indicates a recent history of intermittent constipation. (B) Gene schema of ACTG2 with deletion indicated (red bar). The deletion harbored by the patient corresponds to part of exon 6 of 9, and part of intron 6. (C) Representation of whole-genome sequencing reads overlapping the intragenic ACTG2 deletion (red bar) for the proband (P) and mother (M). Colored nucleotides indicate bases that are mismatched with respect to the reference genome, because of the deletion. (D) Schema of the deletion (red bar) at the single base level, with the 24 deleted base pairs of exon 6 shown.

et al. 2015; Lu et al. 2016). However, hematoxylin and eosin (H&E) staining of this patient’s ileostomy resection revealed neither longitudinal muscle thinning nor smooth muscle fiber disorganization (Fig. 1B).

Treatment Outcomes

Following molecular diagnosis, the patient was started on augmentin (10 mg/kg/dose BID) and erythromycin ethylsuccinate (EES) (3 mg/kg/dose four times daily [QID]), with EES replaced by cisapride (0.1mg/kg/dose QID) following insurance approval. Gut function returned postileostomy takedown and exploratory laparotomy. Per os (PO) feeds were trialed and up-titrated as tolerated, along with TPN. Eventually the patient progressed to PO formula (PediaSure), purees, and solids, and TPN was discontinued. Because of

Table 1. Variant table

Gene	Chromosomal variant coordinate (build 37.1)	HGVS DNA reference	HGVS protein reference	Variant type	Predicted effect	dbSNP ID	Genotype	ClinVar ID
ACTG2	Chr 2:74140749-74140936	c.589_613+163del188	p.?	Deletion	Frameshift	n/a	Homozygous	872896

Table 2. Proband genome sequencing metrics

Metric	Value
Read length	100
Total reads	1,392,792,700
Total mapped reads	1,372,429,438
Nucleotide variants identified	4,913,732
Variants with quality scores >40	94.36%
Coding nucleotide variants identified	25,501
Homozygous: heterozygous ratio of coding nucleotide variants	0.62
Transition: transition ratio of coding nucleotide variants	2.86
Mean coverage	39.5%
Coverage at location of diagnostic variant	36

advancement of oral feeds and stable weight gain, the patient was discharged after a 56-d hospital stay at a discharge weight of 8.18 kg (<1%ile, -2.3 SD), per CDC U.S. growth charts.

He was readmitted 1.5 mo later with increased abdominal distension and nonbilious emesis despite regular stooling. Decompression was achieved with an NG tube and rectal tube along with Pedialax and fleet enemas. The GI motility specialist recommended increasing the cisapride dosage to 0.2 mg/kg/dose QID. Despite these interventions, the patient continued to have worsening abdominal distension and bilious emesis. The decision was made to proceed with an exploratory laparotomy, lysis of adhesions, redo end ileostomy with mucus fistula creation, and gastrostomy tube placement. The patient remained hospitalized for 6.5 mo, as a result of difficulty advancing enteral feeds to goal rate, cycling between vomiting and distension and increased ostomy output. He continues to be followed clinically by several specialties and is noted to experience moderate urinary retention not requiring catheterization.

DISCUSSION

In contrast to numerous reported cases of AD ACTG2-VM, we report here a case of severe visceral myopathy with apparent AR inheritance. Whereas nearly all reported patients with AD ACTG2-VM carry heterozygous missense variants in the ACTG2 gene, this patient carries a homozygous deletion predicted to result in complete loss of functional ACTG2 protein. Two other kindreds with suspected AR ACTG2-associated visceral myopathy have been reported to date, one carrying homozygous LOF variant p.(Arg168*) and the other carrying a homozygous missense variant p.(Arg336Gln). (Monies et al. 2017; Matera et al. 2021). In addition, there is one reported ACTG2-VM case with a deletion of exons 8–9 for which zygosity was not stated (Lee et al. 2019).

The clinical presentation of this patient is comparable to the severe end of the spectrum of AD ACTG2-VM, in which there is evidence of incomplete penetrance and variable intra-familial expressivity, as well as some concordance between specific recurrent variants and particularly poor clinical outcomes (Lehtonen et al. 2012; Wangler et al. 2014; Klar et al. 2015; Matera et al. 2016; Milunsky et al. 2017; Assia Batzir et al. 2019). In AD ACTG2-VM, many patients are symptomatic at birth; 58% of patients undergo abdominal surgery within the first few weeks of life and 44% of patients have a poor outcome defined as reliance on TPN, visceral transplantation, or death in early childhood (Assia Batzir et al. 2019). In contrast,

this patient was reportedly healthy until 3 mo of age but has since undergone numerous hospitalizations and several surgeries and relied on TPN sporadically. Ninety-three percent of patients with AD ACTG2-VM experience urine retention and megacystis requiring catheterization (Assia Batzir et al. 2019). This patient has a history of moderate urinary retention, which has not required catheterization. The family history for this patient was notable only for intermittent constipation in one maternal half-sibling who has not had ACTG2 genetic testing to our knowledge. The mother denies any personal history of GI issues including diarrhea, constipation, vomiting, bloating, or recurrent abdominal pain for herself and the patient's father.

In one reported case of AR ACTG2-VM, two affected siblings carrying a homozygous p.(Arg336Gln) variant presented with severe CIPO and megacystis, whereas heterozygote parents were unaffected (Matera et al. 2021). In another reported case of AR ACTG2-VM, the severely affected patient carrying a homozygous p.(Arg168*) variant presented with CIPO but no urinary symptoms (Monies et al. 2017). This patient had one presumed homozygote sibling who died of intestinal obstruction and perforation, one mildly symptomatic homozygote sibling, and several heterozygote family members reported chronic intermittent constipation (Monies et al. 2017). Taken together, these cases suggest that AR ACTG2-VM is associated with variable expression, and that heterozygote relatives may also exhibit visceral myopathy with variable expression and incomplete penetrance.

The allelic heterogeneity of ACTG2 resembles the pattern reported for ACTA1, which encodes skeletal muscle α -actin 1 and is associated with a spectrum of myopathies. AD ACTA1-related myopathies are overwhelmingly caused by missense variants, many of which have been shown to act in a dominant negative fashion to impair overall assembly, stability, or contraction of actin polymers, whereas AR ACTA1-related myopathies are caused by loss-of function variants (Feng and Marston 2009; Laing et al. 2009). Functional testing of several recurrent heterozygous missense ACTG2 variants has shown that they impair actin multimer assembly and contraction, supporting a dominant negative mechanism (Lehtonen et al. 2012; Thorson et al. 2014). For ACTG2-VM, the report of an AR case associated with homozygous missense variants, and the presence of symptomatic heterozygote carrier relatives in at least one AR case complicates this picture. Ascertainment of additional AR ACTG2-VM cases and functional testing of more pathogenic ACTG2 variants is required for a complete understanding of the spectrum of ACTG2-VM and its molecular pathogenesis.

The pathogenic deletion reported in this patient was discovered through whole-genome sequencing, which is unparalleled for scope of variant discovery (Belkadi et al. 2015). Exome sequencing can generate uneven coverage across and within exons, complicating CNV discovery, and low or absent intronic coverage may prevent detection of intronic breakpoints such as the 3' breakpoint reported here. A 187-bp deletion is beyond the lower limit of detection by even the highest coverage single-nucleotide polymorphism (SNP) arrays. In this case, a rapid molecular diagnosis provided guidance for drug trialing, obviated the need for further diagnostic workup such as tissue biopsies or radiologic studies, and informed genetic counseling. Furthermore, this result supports the allelic heterogeneity of ACTG2-related VM, which may now merit attention as a potential diagnosis in cases in which parental consanguinity is reported.

METHODS

Consent and Enrollment

Parental consent was obtained for this study through Rady Children's Hospital, San Diego.

Genetic Sequencing and Analysis

Following DNA isolation from whole blood, sequencing libraries were generated using TruSeq kits (Illumina). Whole-genome sequencing was performed on NovaSeq 6000 instruments (Illumina), generating paired 101-nt reads with an average coverage of 39.5× for the proband and 52.9× for the mother. Alignment and SNV calling were performed using the DRAGEN hardware and software platform, yielding 4,913,732 calls for the proband and 5,041,148 for the mother.

CNV calling was performed with CNVnator and Manta (Abyzov et al. 2011; Chen et al. 2016). Variant call format (VCF) files incorporating SNV and CNV calls were annotated and analyzed using Fabric Enterprise version 6.5.0 (Fabric Genomics) according to standard guidelines (Coonrod et al. 2013; Richards et al. 2015).

Multiplex ligation-dependent probe amplification (MLPA) was performed on the patient and parental samples and three unrelated control samples, using two custom-designed probes falling within the deleted region. The MLPA products were separated using a 3500 Genetic Analyzer (ThermoFisher), and the results were analyzed using Coffalyser software (MRC-Holland). For each probe, patient to averaged control peak intensity ratios of 0.40–0.65 were considered indicative of a heterozygous deletion, and ratios of 0 were considered indicative of a homozygous deletion.

Histopathology

Full-thickness specimens obtained through small intestinal (ileostomy site) biopsies were formalin fixed, and H&E staining was performed.

ADDITIONAL INFORMATION

Data Deposition and Access

Neither sharing of sequencing data for this patient nor its upload to public repositories is possible, because of parental opt-out during consenting. The ClinVar ID for the ACTG2 deletion reported in this patient is VCV000872896.1.

Ethics Statement

Parental consent for duo whole-genome sequencing was obtained in the course of clinical care. Written consent for publication of clinical details was obtained via a Rady Children's Hospital San Diego (RCHSD) release form, reviewed by the RCHSD Privacy Board, in accordance with RCHSD rules for case reports.

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Author Contributions

K.N.J., M.L., and K.A.E. conceived the study design; K.S., J.L., R.M., R.I., J.H., L.C., and S.K. acquired and analyzed the clinical data; K.N.J. and M.L. drafted the manuscript; and K.N.J., M.L., R.I., S.K., and K.A.E. revised the manuscript for intellectual content.

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Competing Interest Statement

The authors have declared no competing interest.

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