Published in final edited form as:

Int J Surg Pathol. 2019 February ; 27(1): 77–83. doi:10.1177/1066896918786586.

ACTG2 associated visceral myopathy with chronic intestinal pseudo-obstruction, intestinal malrotation, hypertrophic pyloric stenosis, choledochal cyst, and a novel missense mutation

Rebecca R. J. Collins, MD^{1,2}, Bradley Barth, MD, MPH^{1,2}, Stephen Megison, MD^{1,2}, Cory M. Pfeifer, MD^{1,2}, Luke M. Rice, PhD¹, Samar Harris, MD¹, Charles F. Timmons, MD, PhD^{1,2}, Dinesh Rakheja, MD^{1,2}

¹University of Texas Southwestern Medical Center, Dallas, Texas, USA.

²Children's Health, Dallas, Texas, USA

Abstract

Primary visceral myopathy caused by a pathogenic mutation in the gene encoding the enteric smooth muscle actin gamma 2 (*ACTG2*) affects gastrointestinal and genitourinary tracts and often presents as chronic intestinal pseudoobstruction. We present a case of pediatric onset chronic intestinal pseudoobstruction associated with a novel missense *ACTG2* mutation c.439G>T / p.G147C. In addition to the known disease manifestations of feeding intolerance and intestinal malrotation, our patient had a late-onset hypertrophic pyloric stenosis and a late-onset choledochal cyst, the former of which has not previously been described in patients with *ACTG2* associated visceral myopathy.

Keywords

ACTG2; Actin gamma 2; Chronic intestinal pseudoobstruction; Choledochal cyst; CIPO; Hypertrophic pyloric stenosis; Visceral myopathy

Introduction

Chronic intestinal pseudoobstruction (CIPO) is an etiologically, pathologically, and clinically heterogeneous syndrome defined by ineffective intestinal peristalsis in the absence of a mechanical obstruction. CIPO is a manifestation of gastrointestinal neuromuscular pathology that may result from a primary developmental or genetic disorder or from secondary involvement by a systemic disorder such as diabetes, amyloidosis, or collagen vascular disease.^{1–3} The primary gastrointestinal neuromuscular disorders are more likely to present in childhood; therefore, the term pediatric intestinal pseudoobstruction (PIPO) has recently been proposed to distinguish the pediatric onset disorders from adult onset CIPO.⁴ Pathogenic mutations in the gene encoding the enteric smooth muscle contractile protein actin gamma 2 (*ACTG2*) are associated with a primary visceral myopathy that often presents

with pediatric onset CIPO.⁵ Here we describe a patient with pediatric onset CIPO associated with a novel missense germline *ACTG2* mutation and unusual disease manifestations of hypertrophic pyloric stenosis and choledochal cyst.

Case report

A male patient presented to our hospital at the age of 12 years for a second opinion and management of his symptoms of chronic intestinal obstruction. Prior to this, he had been managed for feeding intolerance since early infancy at an outside institution. He had undergone laparotomies for correction of intestinal malrotation and lysis of intestinal adhesions. More recently, he had developed persistent vomiting and poor gastric emptying secondary to pyloric obstruction, for which he had received pyloric dilations, botulinum toxin injections, total parenteral nutrition, and a gastrostomy. At our hospital, an abdominal radiograph showed a massively dilated stomach (Figure 1A). A laparotomy was performed and an 8 cm × 5 cm pyloric mass (Figure 1B) was found obstructing the gastric outlet. Additionally, there were dilated small bowel loops without an obstructing lesion. The pyloric mass was resected and a primary gastroduodenostomy was performed. The patient improved clinically and his gastrostomy was taken down. Three years later, he presented with abdominal pain and distension with oral intake and resultant failure to thrive. Magnetic resonance cholangiopancreatography showed the common bile duct to be dilated to 2.3 cm in diameter with extension of ductal dilation into the intrahepatic ducts (Figure 2). Subsequent imaging demonstrated persistently dilated small bowel loops. He underwent a choledochal cyst resection, along with a cholecystectomy and a full-thickness biopsy of the duodenum. Due to persistent failure to thrive, he was started on total parenteral nutrition. He has also been on a regimen of cyclical antibiotics for treatment of small intestinal bacterial overgrowth that may cause mucosal inflammation further impairing gastrointestinal motility.⁶ Recently, he has been started on a trial of the enterokinetic pyridostigmine with mild relief of bloating.⁷

Pathologic Findings

Gross pathologic examination of the pyloric mass showed a diffuse thickening of the pyloric wall (Figures 1C). Histopathologic examination showed hyperplastic smooth muscle of the muscularis propria (Figure 1D) and the possibility of late-onset idiopathic hypertrophic pyloric stenosis was considered. In retrospect, occasional eosinophilic inclusions (Figure 1D), better demonstrated by immunohistochemical staining for smooth muscle actin (Figure 1E) were present in the cytoplasm of the smooth muscle cells. The full-thickness duodenal biopsy showed unremarkable mucosa and submucosa, normal appearing myenteric and submucosal neural plexuses, and normal-appearing distribution of interstitial cells of Cajal as demonstrated by a CD117 immunostain. While the muscularis mucosae appeared unremarkable, the smooth muscle cells in the muscularis propria appeared disarrayed. Hematoxylin and eosin stained sections suggested eosinophilic inclusions in the cytoplasm of the smooth muscle cells of the muscularis propria (Figure 3A). These ovoid to irregular inclusions did not stain with periodic acid Schiff stain (Figure 3B), but stained purple with Masson trichrome stain (Figure 3C) and pale blue with toluidine blue stain (Figure 3D). Immunostains for smooth muscle actin (Figure 3E) and muscle specific actin highlighted the

inclusions. By transmission electron microscopy, the inclusions corresponded to irregular aggregates of 9–11 nm filaments (Figures 3F). Immunostain for smooth muscle actin showed similar inclusions in the smooth muscle cells in the walls of the gallbladder and the choledochal cyst.

Molecular Findings

Deoxyribonucleic acid (DNA) was extracted from formalin fixed and paraffin embedded (FFPE) tissue (QIAamp DNA FFPE Tissue Kit, Qiagen, Hilden, Germany). The entire coding region of the *ACTG2* gene was amplified using previously published primers.⁸ Sanger sequencing identified a heterozygous missense mutation c.439G>T that results in replacement of the glycine at position 147 with cysteine (p.G147C, Figures 4A–4B). The mutation was also confirmed in the DNA extracted from the patient's peripheral blood. This mutation has previously not been described (Table 1).

Discussion

Mutations in the *ACTG2* gene cause an autosomal dominant familial visceral myopathy that involves the intestines, the urinary bladder, and/or the uterus.^{5, 9} The severity of the disease is variable. At the severe end of the spectrum, the disease may present at birth with megacystic microcolon intestinal hypoperistalsis syndrome (MMIHS), microcolon, megacystis and megaureter, and prune-belly syndrome. Other clinical presentations include recurrent urinary tract infections, bladder dysfunction, intestinal malrotation, feeding intolerance, cholecystitis/ cholelithiasis, and CIPO.⁵ While *ACTG2* associated visceral myopathy causes intestinal pseudoobstruction, mechanical intestinal obstruction may also occur due to adhesions secondary to abdominal surgeries. However, mechanical obstruction due to hypertrophic pyloric stenosis as seen in our patient has not previously been described in *ACTG2* associated visceral myopathy. We posit that hypertrophic pyloric stenosis seen in our patient is not an incidental finding, but a feature of *ACTG2* associated visceral myopathy because of the presence of smooth muscle actin-positive inclusions in the cytoplasm of the smooth muscle cells of the hypertrophied pyloric muscularis propria. Additional support for this association comes from the reports of hypertrophic pyloric stenosis in association with CIPO, intestinal malrotation, and short bowel in patients with X-linked periventricular nodular heterotopia caused by mutations in the gene encoding Filamin A (*FLNA*), which is an actin binding protein that regulates actin cytoskeleton.^{10–12} The absence of hypertrophic pyloric stenosis in the previously reported cases of *ACTG2* associated visceral myopathy suggests that the previously undescribed *ACTG2* mutation, c.439G>T / p.G147C, seen in our patient may be specifically associated with hypertrophic pyloric stenosis and/or there may be other unidentified genetic or epigenetic modifiers of the phenotype. Another unusual manifestation in our case was the acquired choledochal cyst first noted when the patient was 12 years old. Pertinently, smooth muscle positive inclusions were present in the cytoplasm of the smooth muscle cells of the choledochal cyst. Previously, Lu et al reported choledochal cyst in one infant with *ACTG2* associated MMIHS.¹³

The diagnosis of *ACTG2* associated visceral myopathy is established in a patient by the presence of a heterozygous mutation in *ACTG2*.⁵ The *ACTG2* mutation, c.439G>T / p.G147C, seen in our patient has not previously been described. Including this mutation, there are now 23 known pathogenic variants of *ACTG2* associated visceral myopathy (Table 1).^{8, 9, 13–24} Twenty-two of these are missense mutations with one being a heterozygous tandem base substitution c.806_807delinsAA (p.G269E) reported in seven affected members of a Swedish family.⁹ In addition to these 23 mutations in patients with visceral myopathy, Wangler et al identified an incidental nonsense variant (c.C187T, p.R63X) in *ACTG2* by exome sequencing a group of approximately 1900 individuals. The individual with this *ACTG2* variant reported intermittent mild constipation that did not require medications, and had no history of abdominal surgery or bladder dysfunction. The authors concluded that *ACTG2* haploinsufficiency is not clinically significant but cautioned that a mild phenotype with incomplete penetrance could not be excluded.²² In in vitro studies, the pathogenic variants of *ACTG2* show impaired polymerization and reduced incorporation into actin filaments, and their presence leads to reduced cell contractility.^{8, 15, 18} In sarcoma cells, exogenous *ACTG2*-R148S interfered with endogenous actin cytoskeletal organization and cell contractility suggesting a dominant negative effect of the mutant protein.¹⁸ The novel mutation, p.G147C, seen in our patient affects the glycine that lies in a cleft that forms the binding site for the Wiskott-Aldrich syndrome homology region 2 (WH2)-containing proteins and other actin regulatory factors (Figure 5A), and that is also important for actin:actin contacts in the filament.^{25, 26} This would explain the ultrastructural finding of disorganized actin filament aggregates and the light microscopic finding of actin-positive inclusions in the smooth muscle cells. Further, the *ACTG2* protein sequence, including the G147 residue is evolutionarily highly conserved (Figure 5B). The mutation, c.439G>T / p.G147C, is predicted to be *probably damaging* by the computation tool PolyPhen-2²⁷ (score 0.998), *damaging* by the computation tool SIFT²⁸ (score 0.01), and *deleterious* by the computation tool PROVEAN²⁹ (score -7.12).

The reported histopathologic and immunohistochemical findings in *ACTG2* associated visceral myopathy have been variable. Lehtonen et al reported *ACTG2*-positive intracellular inclusions as well as reduced *ACTG2* staining in smooth muscle cells throughout the enteric muscularis propria in four affected members of a Finnish family.¹⁸ Matera et al reported severe atrophy of muscularis propria, reduced connective fiber network, and large ganglia in one 7-year old patient with *ACTG2* associated CIPO.¹⁹ In four patients with *ACTG2* associated MMIHS, Lu et al reported loose and disordered arrangement of intestinal smooth muscle cells of the muscularis propria by light microscopy and vacuolar degeneration in the smooth muscle cells by transmission electron microscopy.¹³ In a neonate with *ACTG2* associated MMIHS, Korgali et al reported atrophic smooth muscle cells with attenuated muscularis propria in the small intestine; however, immunoreactivity for smooth muscle actin was preserved in the smooth muscle cells. Masson trichrome stain showed mural fibrosis and an immunostain for CD117 showed fewer interstitial cells of Cajal compared to an age-matched control.¹⁷ In a 55-year-old female with *ACTG2* associated CIPO, Holla et al reported haphazardly arranged and swollen smooth muscle cells with decreased immunohistochemical expression of smooth muscle actin in the muscularis propria and lack of separation between the inner circular and the outer longitudinal layers. There was also

disordered myenteric neural plexus highlighted by S100 immunostain. The intestinal pathology specimen of that patient's deceased brother, initially suspected to have Crohn's disease, showed similar findings.¹⁶ In one patient with a heterozygous tandem base substitution c.806_807delinsAA (p.G269E) in *ACTG2*, Klar et al reported normal light microscopic appearance of a full-thickness biopsy from the distal ileum and cecum with normal immunohistochemical staining for smooth muscle actin.⁹ Histologic examination in our patient showed haphazardly arranged smooth muscle cells with smooth muscle actin-positive inclusions in the muscularis propria of the duodenum. Similar inclusions were also seen in the smooth muscle cells of the hypertrophic pyloric stenosis and in the walls of the gallbladder and the choledochal cyst. It is not yet clear if the variability in the reported histopathologic and immunohistochemical findings in *ACTG2* associated visceral myopathy have a correlation with the underlying mutations or if there are additional genetic or epigenetic factors at play.

In summary, we have described a novel missense *ACTG2* mutation in a patient with CIPO and unusual disease manifestations of late-onset hypertrophic pyloric stenosis and choledochal cyst, thereby expanding the genetic, clinical, and pathologic phenotype of this uncommon disorder.

References

1. Knowles CH, De Giorgio R, Kapur RP, Bruder E, Farrugia G, Geboes K, et al. The London Classification of gastrointestinal neuromuscular pathology: report on behalf of the Gastro 2009 International Working Group. *Gut*. 2010;59:882–887. [PubMed: 20581236]
2. Knowles CH, De Giorgio R, Kapur RP, Bruder E, Farrugia G, Geboes K, et al. Gastrointestinal neuromuscular pathology: guidelines for histological techniques and reporting on behalf of the Gastro 2009 International Working Group. *Acta Neuropathol*. 2009;118:271–301. [PubMed: 19360428]
3. Di Nardo G, Di Lorenzo C, Lauro A, Stanghellini V, Thapar N, Karunaratne TB, et al. Chronic intestinal pseudo-obstruction in children and adults: diagnosis and therapeutic options. *Neurogastroenterol Motil*. 2017;29.
4. Thapar N, Saliakellis E, Benninga MA, Borrelli O, Curry J, Faure C, et al. Paediatric Intestinal Pseudo-Obstruction: Evidence and Consensus-Based Recommendations from an ESPGHAN-Led Expert Group. *J Pediatr Gastroenterol Nutr*. 2018.
5. Wangler MF, Beaudet AL. *ACTG2-Related Disorders*. In: Pagon RA, Adam MP, Ardinger HH, et al., eds. *GeneReviews(R)* Seattle (WA); 2015.
6. Husebye E. Gastrointestinal motility disorders and bacterial overgrowth. *J Intern Med*. 1995;237:419–427. [PubMed: 7714466]
7. O'Dea CJ, Brookes JH, Wattchow DA. The efficacy of treatment of patients with severe constipation or recurrent pseudo-obstruction with pyridostigmine. *Colorectal Dis*. 2010;12:540–548. [PubMed: 19508545]
8. Thorson W, Diaz-Horta O, Foster J 2nd, Spiliopoulos M, Quintero R, Farooq A, et al. De novo *ACTG2* mutations cause congenital distended bladder, microcolon, and intestinal hypoperistalsis. *Hum Genet*. 2014;133:737–742. [PubMed: 24337657]
9. Klar J, Raykova D, Gustafson E, Tothova I, Ameer A, Wanders A, et al. Phenotypic expansion of visceral myopathy associated with *ACTG2* tandem base substitution. *Eur J Hum Genet*. 2015;23:1679–1683. [PubMed: 25782675]
10. Oegema R, Hulst JM, Theuns-Valks SD, van Unen LM, Schot R, Mancini GM, et al. Novel no-stop *FLNA* mutation causes multi-organ involvement in males. *Am J Med Genet A*. 2013;161A:2376–2384. [PubMed: 23873601]

11. Nezelof C, Jaubert F, Lyon G. [Familial syndrome combining short small intestine, intestinal malrotation, pyloric hypertrophy and brain malformation. 3 anatomoclinical case reports]. *Ann Anat Pathol (Paris)*. 1976;21:401–412. [PubMed: 1023783]
12. Peeters B, Benninga MA, Hennekam RC. Infantile hypertrophic pyloric stenosis--genetics and syndromes. *Nat Rev Gastroenterol Hepatol*. 2012;9:646–660. [PubMed: 22777173]
13. Lu W, Xiao Y, Huang J, Tao Y, Yan W, Lu L, et al. Mutation in Actin gamma-2 Responsible for Megacystis Microcolon Intestinal Hypoperistalsis Syndrome in 4 Chinese Patients. *J Pediatr Gastroenterol Nutr*. 2016;63:624–626. [PubMed: 27007401]
14. Farwell KD, Shahmirzadi L, El-Khechen D, Powis Z, Chao EC, Tippin Davis B, et al. Enhanced utility of family-centered diagnostic exome sequencing with inheritance model-based analysis: results from 500 unselected families with undiagnosed genetic conditions. *Genet Med*. 2015;17:578–586. [PubMed: 25356970]
15. Halim D, Hofstra RM, Signorile L, Verdijk RM, van der Werf CS, Sribudiani Y, et al. ACTG2 variants impair actin polymerization in sporadic Megacystis Microcolon Intestinal Hypoperistalsis Syndrome. *Hum Mol Genet*. 2016;25:571–583. [PubMed: 26647307]
16. Holla OL, Bock G, Busk OL, Isfoss BL. Familial visceral myopathy diagnosed by exome sequencing of a patient with chronic intestinal pseudo-obstruction. *Endoscopy*. 2014;46:533–537. [PubMed: 24777424]
17. Korgali EU, Yavuz A, Simsek CEC, Guney C, Kurtulgan HK, Baser B, et al. Megacystis Microcolon Intestinal Hypoperistalsis Syndrome in Which a Different De Novo Actg2 Gene Mutation was Detected: A Case Report. *Fetal Pediatr Pathol*. 2018;37:109–116. [PubMed: 29608093]
18. Lehtonen HJ, Sipponen T, Tojkander S, Karikoski R, Jarvinen H, Laing NG, et al. Segregation of a missense variant in enteric smooth muscle actin gamma-2 with autosomal dominant familial visceral myopathy. *Gastroenterology*. 2012;143:1482–1491 e1483. [PubMed: 22960657]
19. Matera I, Rusmini M, Guo Y, Lerone M, Li J, Zhang J, et al. Variants of the ACTG2 gene correlate with degree of severity and presence of megacystis in chronic intestinal pseudo-obstruction. *Eur J Hum Genet*. 2016;24:1211–1215. [PubMed: 26813947]
20. Moreno CA, Metze K, Lomazi EA, Bertola DR, Barbosa RH, Cosentino V, et al. Visceral myopathy: Clinical and molecular survey of a cohort of seven new patients and state of the art of overlapping phenotypes. *Am J Med Genet A*. 2016;170:2965–2974. [PubMed: 27481187]
21. Tuzovic L, Tang S, Miller RS, Rohena L, Shahmirzadi L, Gonzalez K, et al. New Insights into the Genetics of Fetal Megacystis: ACTG2 Mutations, Encoding gamma-2 Smooth Muscle Actin in Megacystis Microcolon Intestinal Hypoperistalsis Syndrome (Berdon Syndrome). *Fetal Diagn Ther*. 2015;38:296–306. [PubMed: 25998219]
22. Wangler MF, Gonzaga-Jauregui C, Gambin T, Penney S, Moss T, Chopra A, et al. Heterozygous de novo and inherited mutations in the smooth muscle actin (ACTG2) gene underlie megacystis-microcolon-intestinal hypoperistalsis syndrome. *PLoS Genet*. 2014;10:e1004258. [PubMed: 24676022]
23. Whittington JR, Poole AT, Dutta EH, Munn MB. A Novel Mutation in ACTG2 Gene in Mother with Chronic Intestinal Pseudoobstruction and Fetus with Megacystis Microcolon Intestinal Hypoperistalsis Syndrome. *Case Rep Genet*. 2017;2017:9146507. [PubMed: 29387497]
24. Stark Z, Tan TY, Chong B, Brett GR, Yap P, Walsh M, et al. A prospective evaluation of whole-exome sequencing as a first-tier molecular test in infants with suspected monogenic disorders. *Genet Med*. 2016;18:1090–1096. [PubMed: 26938784]
25. Chereau D, Kerff F, Graceffa P, Grabarek Z, Langsetmo K, Dominguez R. Actin-bound structures of Wiskott-Aldrich syndrome protein (WASP)-homology domain 2 and the implications for filament assembly. *Proc Natl Acad Sci U S A*. 2005;102:16644–16649. [PubMed: 16275905]
26. Dominguez R, Holmes KC. Actin structure and function. *Annu Rev Biophys*. 2011;40:169–186. [PubMed: 21314430]
27. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010;7:248–249. [PubMed: 20354512]

28. Sim NL, Kumar P, Hu J, Henikoff S, Schneider G, Ng PC. SIFT web server: predicting effects of amino acid substitutions on proteins. *Nucleic Acids Res.* 2012;40:W452–457. [PubMed: 22689647]
29. Choi Y, Sims GE, Murphy S, Miller JR, Chan AP. Predicting the functional effect of amino acid substitutions and indels. *PLoS One.* 2012;7:e46688. [PubMed: 23056405]

Figure 1.

A. Abdominal radiograph shows a markedly dilated stomach (yellow arrows). A previously placed gastrojejunostomy tube (red arrow) had retracted into the gastric antrum. B. At laparotomy, a pyloric mass (yellow arrow) and dilated loops of small bowel (red arrows) were noted. C. Gross examination of the resected pyloric mass shows diffuse mural thickening. D. Light microscopic examination shows diffuse smooth muscle hyperplasia consistent with hypertrophic pyloric stenosis; occasional eosinophilic intracytoplasmic irregular inclusions can be seen (hematoxylin and eosin, 400x total magnification). E. Immunohistochemical staining shows smooth muscle actin-positive irregular inclusions in the cytoplasm of smooth muscle cells of the muscularis propria (400x total magnification).

Figure 2. Maximum intensity projection from 3D-T2-weighted magnetic resonance cholangiopancreatography shows fusiform dilatation of the common bile duct (red arrow) with dilated intrahepatic biliary ducts (yellow arrow).

Figure 3.

Photomicrographs of full-thickness duodenal biopsy show haphazardly arranged smooth muscle cells in the muscularis propria with eosinophilic intracytoplasmic irregular inclusions (A. hematoxylin and eosin, 400x total magnification) that do not stain with periodic acid Schiff (B. 400x total magnification) and stain purple with Masson trichrome (C. 400x total magnification) and pale blue with toluidine blue (D. 400x total magnification). The inclusions are smooth muscle actin-positive (E. immunoperoxidase, 400x total magnification) and ultrastructurally composed of irregular aggregates of 9–11 nm filaments (F. 1500x total magnification).

Figure 4.

A. Sanger sequencing of the entire coding region of the *ACTG2* gene identified a novel heterozygous missense mutation c.439G>T that results in replacement of the glycine at position 147 with cysteine. B. A cartoon of the exons (numbered 1–9) and coding region (in gray) of the *ACTG2* gene shows the location of the reported mutations associated with visceral myopathy.

Figure 5.
A. A cartoon representation showing the structural context for the mutated site. Actin (pale blue) and a bound WH2 domain from WASP (tan) from Protein Data Bank entry 2A3Z (<https://www.rcsb.org/structure/2A3Z>) are shown, with ATP as sticks. The position of the mutated site is indicated with a red sphere. B. A comparison across species shows that the ACTG2 protein sequence including the G147 residue (arrow) is evolutionarily conserved (Alamut Visual, Interactive Biosoftware, Rouen, France).

TABLE 1.*ACTG2* mutations associated with visceral myopathy

Amino acid change	References
p.A23T	https://www.ncbi.nlm.nih.gov/clinvar/RCV000585758/
p.R38H	9
p.R40C	15, 22
p.R40H	22
p.M45T	22
p.R63G	22
p.R63Q	15
p.F110L	22
p.Y134N	22
p.G147C	Current case
p.R148S	16, 18, 19
p.R148L	https://www.ncbi.nlm.nih.gov/clinvar/RCV000157596/
p.R178C	8, 15, 19, 20, 22
p.R178H	14, 15, 19, 21, 22
p.R178L	8, 15, 19, 20
p.R178S	17
p.T195I	20
p.G198D	22
p.A205T	24
p.R211Q	23
p.R257C	13, 14, 19, 21, 22
p.R257H	13, 14, 19–21
p.G269E	9