CME

Diagnosis of Chronic Intestinal Pseudo-obstruction and Megacystis by Sequencing the *ACTG2* Gene

*†Aubrey Milunsky, *Clinton Baldwin, *Xiaoying Zhang, *Daniel Primack, ‡§Adrian Curnow, and *†Jeff Milunsky

ABSTRACT

Objectives: The diagnosis of chronic intestinal pseudo-obstruction has depended on clinical features, manometry, and imaging. This report aimed to determine the efficacy of sequencing the actin γ-2 (*ACTG2*) gene for diagnosis. In addition, the goal was to determine how often a mutation would be found in our randomly collected cohort of probands and those probands published previously.

Methods: Whole exome sequencing was performed in 4 probands with chronic intestinal pseudo-obstruction. Subsequently, only the *ACTG2* gene was sequenced in another 24 probands (total 28). We analyzed published data of 83 probands and our 28 (total 111) and determined how many had pathogenic variants and the precise genotype.

Results: Whole exome and Sanger sequencing revealed a pathogenic variant in the *ACTG2* gene in 4 out of 28 of our probands and in 45 out of 83 published probands (49/111 [44.1%]). Moreover, a mutational hotspot in the *ACTG2* gene was recognized. Genetic heterogeneity is evident.

Conclusions: Pooled gene sequencing results from 1 individual in each of 111 families enabled a precise diagnosis of an *ACTG2* mutation in 49 (44%). The benefit to patients and families of early confirmation of a motility disorder not only helps avoid unnecessary intervention, but also enables institution of appropriate treatments and avoidance of secondary disorders such as malnutrition and poor growth. Knowledge of a pathogenic variant in a parent, with a 50% risk of recurrence, provides an opportunity for genetic counseling.

Key Words: gastrointestinal transplantation, gastroparesis, megaureter., visceral myopathy

(*JPGN* 2017;65: 384–387)

What Is Known

- Chronic intestinal pseudo-obstruction with or without megacystis is a debilitating dominant or recessive disorder with a high mortality rate and a variable phenotype.
- Diagnosis has depended on clinical signs, manometry, and radiology.
- Pathogenic mutations in a single gene have been recognized recently.
- Very few families have had gene sequencing.

What Is New

- Four out of 28 of our probands had an *ACTG2* gene mutation, enabling a precise diagnosis.
- Sequencing of this gene in our 28 probands and 83 published probands shows for the first time that 49 of 111 (44.1%) have an *ACTG2* mutation.
- For the first time, a mutational hotspot is recognized.

Chronic intestinal pseudo-obstruction (CIPO), the nomenclature for which has included megacystis microcolon intestinal hypoperistalsis syndrome and hollow visceral myopathy (1–4). MIM 155310 is a mostly severely debilitating disorder of enteric smooth muscle. Other forms of CIPO may occur as a consequence of mitochondrial disease (5). This is a disorder that frequently demands total parenteral nutrition (TPN) for decades and which may lead eventually to total intestinal, liver, pancreas, and spleen transplantation, or death before such dramatic interventions. The clinical features of this progressive disorder encompass a variable phenotype. Gastrointestinal symptoms and signs due to gastroparesis typically include abdominal distension and pain, nausea, and vomiting. Often, there is a need for abdominal surgery, which may include gastrostomy, ileostomy, intestinal resection, or colectomy. Survivors with intestinal failure may need total gastrointestinal transplantation. Megacystis and megaureter, often evident at birth, may also be detected prenatally by ultrasound imaging. Bladder involvement is almost invariable in all and frequently severe. Lifetime catheterization of the bladder is almost invariable in all who have megacystis despite surgical efforts to limit the size of the bladder. A high mortality rate attends this disorder (6–9) with 1 report indicating a 19.7% survival rate (6).

The genetic basis for CIPO was uncertain until the first Finish report (3) of a family in which an autosomal dominant pathogenic variant in the enteric smooth muscle actin γ-2 (*ACTG2*) gene was determined in adult-onset visceral myopathy. Additional families with pathogenic variants in this gene have subsequently been identified, with 45 probands reported (3,4, 1016) from randomly collected families. Some families, however,

Received July 29, 2016; accepted February 26, 2017.

From the *Center for Human Genetics, Cambridge, the †Department of Obstetrics and Gynecology, Tufts University School of Medicine, Boston, MA, the ‡St. Luke's Regional Medical Center, Boise, ID, and the §University of Washington Center for Mendelian Genomics, Seattle, WA.

Address correspondence and reprint requests to Aubrey Milunsky, MD, DSc, Center for Human Genetics, 840 Memorial Dr, Suite 101, Cambridge, MA 02139 (e-mail: amilunsky@chginc.org).

This article has been developed as a Journal CME Activity by NASPGHAN. Visit *http://www.naspghan.org/content/59/en/Continuing-Medical-Education-CME* to view instructions, documentation, and the complete necessary steps to receive CME credit for reading this article.

The study was supported by Human Genome Research Institute and the National Heart, Lung, and Blood Institute grant U54HG006493.

The authors report no conflicts of interest.

DOI: 10.1097/MPG.0000000000001608

manifest autosomal dominant inheritance but do not have an *ACTG2* mutation, implying that one or more genes remain to be discovered.

This report focuses on 4 probands with *ACTG2* pathogenic variants from 4 families with severe CIPO and megacystis, out of a randomly collected cohort of 28 probands.

METHODS

Four of our probands had whole exome sequencing by the University of Washington Center for Mendelian Genomics, with us analyzing the data provided. After a single pathogenic variant was found in one proband, we proceeded with Sanger sequencing of our other 24 probands with their consent. The present study received institutional review board approval (H-23846/1797B) at Boston University School of Medicine.

Whole Exome Sequencing

Initial quality control entailed DNA quantification, sex typing, and molecular "fingerprinting" using a high-frequency genotyping assay. Library construction and exome capture were automated (Perkin-Elmer Janus II) in a 96-well plate format. One microgram of genomic DNA was subjected to a series of shotgun library construction steps, including fragmentation through acoustic sonication (Covaris), end-polishing and A-tailing, ligation of sequencing adaptors, and polymerase chain reaction (PCR) amplification with 8 bp barcodes for multiplexing. Libraries undergo exome capture using the Roche/Nimblegen SeqCap EZ v2.0 (~36.5 MB target). Before sequencing, the library concentration is determined by triplicate qPCR and molecular weight distributions verified on the Agilent Bioanalyzer (150 ± 15 bp). Barcoded exome libraries were pooled using liquid handling robotics before clustering (Illumina cBot) and loading. Massively parallel sequencing by synthesis with fluorescently labeled, reversibly terminating nucleotides was carried out on the HiSeq sequencer.

The sequencing analysis pipeline consists of base calling, alignment, local realignment, duplicate removal, quality recalibration, data merging, variant detection, genotyping, and annotation using a combined suite of Illumina software, other software packages (Genome Analysis ToolKit, Picard, BWA-MEM, SAMTools, and in-house custom scripts). Variant detection and genotyping are performed using the HaplotypeCaller tool from Genome Analysis ToolKit (3.2). A variant quality score recalibration on the "raw" variant call file (VCF) is used to generate a filtered VCF call set.

Data quality control included an assessment of (1) total reads (minimum of 50 million PE50 reads); (2) library complexity; (3) capture efficiency; (4) coverage distribution: 90% at 8X required for completion; (5) capture uniformity; (6) raw error rates; (7) transition/transversion ratio (Ti/Tv); (8) distribution of known and novel variants relative to the Database of Short Genetic Variation is typically <7%; (9) fingerprint concordance >99%; (10) sample homozygosity and heterozygosity; and (11) sample contamination validation. Exome completion is defined as having >90% of the exome target at >8X coverage and >80% of the exome target at >20X coverage. Typically this requires mean coverage of the target at 50–60X. The SeattleSeq Annotation Server (*http://gvs.gs.washington.edu/SeattleSeqAnnotation/*) was used to annotate the final VCF file.

Sanger Sequencing

Primers flanking each of the 8 coding exons and flanking intronic regions of *ACTG2* were designed using the Primer3 program (*http://bioinfo.ut.ee/primer3/*). After treatment with ExoSAP-IT, the PCR product was sequenced with the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California) followed by capillary electrophoresis on an ABI 3730 sequencer.

RESULTS

Our patients were randomly recruited over at least 15 years. All had the typical symptoms and signs of CIPO with or without megacystis. These included bloating, abdominal distention and pain, pseudo-obstruction, nausea, vomiting and failure to thrive, and weight loss.

The clinical data for our 4 probands (cases 1, 3, 4, and 5) and their family members (cases 2, 6, and 7) with *ACTG2* pathogenic variants in this report (Table 1) uniformly reflect severe CIPO and megacystis. Megacystis was present prenatally and evident at birth in all 7. Three died at 6 months, 2 years, and 11.5 years of age. One mother at 38 years of age suffering intestinal failure had total visceral exenteration that included her stomach, intestine, liver, pancreas, gall bladder, and spleen followed by multiorgan transplantation. She had required TPN for 35 years, from the age of 3. Four of the 7 needed long-term TPN. Three each had gastrostomy, colectomy, and ileostomy. Because of the megacystis, 6 have endured life-time bladder catheterization.

The parents of 2 of our 4 probands had no *ACTG2* pathogenic variants, in all likelihood reflecting de novo variants.

In those without an *ACTG2* mutation, the noted details reflect only information from the last contact which, for many, was at least 10 years. The onset was apparent prenatally or by 2 years of age in 18 of 24. Twenty-one were girls. Two children died. Eight of 24 had colectomies, 2 of 24 had malrotation or volvulus, 5 of 24 had ileostomy or jejunostomy or cecostomy, 10 of 24 needed TPN (1 for 29 years), and 11 of 24 had megacystis or urinary retention. All were Caucasians. All had manometry (dysmotility patterns not known by us) and/or endoscopy.

DISCUSSION

An autosomal dominant mode of inheritance with complete penetrance is clear in the familial cases with *ACTG2* pathogenic variants. Autosomal recessive inheritance of CIPO is highly likely in children of consanguineous unions (17,18) or in affected siblings with healthy parents (bearing in mind gonadal mosaicism). A homozygous pathogenic variant in the RAD21 gene was reported in one consanguineous family (18). Four of 28 of our cohort and 15 of 27 in the report by Wangler et al (4) were found to harbor an *ACTG2* pathogenic variant. The difference between these 2 randomly collected cohorts remains unexplained, but may reflect inaccurate clinical diagnoses.

Of the reported 45 probands and our 4 with CIPO, 33 of 49 (73.3%) have pathogenic variants at either amino acid R178 or R257. The most common pathogenic variants observed, R178C and R257C, involve a C>T transition at CpG dinucleotides (Fig. 1). Methylated CpG sequences frequently undergo mutation caused by random deamination of 5-methycytosines leading to a C>T transition. Thus, the 2 amino acids, R178 and R257, are likely mutational hotspots in *ACTG2*.

Thus far, all pathogenic variants detected in the *ACTG2* gene have been missense variants with no exon or whole gene deletions/duplications being reported. These variants lead to changes in protein function, impair *ACTG2* polymerization, and contribute to reduced smooth muscle cell contractility (10). Although pathogenic variants in recessive and mitochondrial genes that cause CIPO and or bladder involvement are known, they are invariably associated with disorders or defects in other organ systems (19). From our 4 probands, together with 45 other published molecularly confirmed probands, a few early insights are emerging, but further gene discovery is awaited.

TABLE 1. Clinical data of 4 probands (1, 3, 4, and 5) and 3 affected family members (2-A, 6-B, and 7-B) with chronic intestinal pseudo-obstruction and megacystis

Clinical data	Case 1-A	Case 2-A	Case 3	Case 4	Case 5-B	Case 6-B	Case 7-B
ACTG2 Mutation	R257C	R257C	R257C	R257C	R40H	R40H*	R40H*
Current age	39	6	Died	Died	3	Died	28
Age of onset	Birth	Prenatal	Prenatal	Birth	Birth	Prenatal	Birth
Age of death	–	–	2	11.5	–	6 mo	–
Sex	F	F	F	M	M	M	M
Parent or sib affected	–	+	–	–	+	+	–
Pseudo-obstruction	+	+	+	+	+	+	+
Colectomy	+	+	–	–	–	–	+
Ileostomy	+	+	–	–	–	–	+
Cholecystectomy	+	–	–	–	–	–	–
Cholelithiasis	+	–	–	–	–	–	–
Microcolon	–	–	–	–	–	–	–
Gastrostomy	+	+	–	+	–	–	–
Nissen fundoplication	+	+	–	–	–	–	–
Small bowel resection	+	+	–	–	–	–	–
Malrotation/volvulus	–	–	+	+	–	–	–
GE reflux	+	+	+	+	–	–	–
Cirrhosis/liver failure	+	–	–	+	–	–	–
Long-term TPN dependence	35 y	6 y	+	+	–	–	–
Organ transplantation	+	–	–	–	–	–	–
Megacystis	+	+	+	+	+	+	+
Long-term bladder catheterization	–	+	+	+	+	+	+
Hydronephrosis	+	–	–	–	+	+	–
Premature labor	+	–	N/K	+	–	+	+
Other medical disorders	Thrombosis inferior vena cava /renal vein	VSD	–	Pancreatitis seizures	–	–	N/K

– = No; + = yes; A = family A; B = family B; N/K = not known; TPN = total parenteral nutrition; VSD = ventricular septal defect.
*By inference.

The highly variable phenotype and the few that have had molecular studies have made a reliable estimate of the incidence or prevalence of CIPO with or without megacystis (20) difficult to determine. The realization that a proven affected parent may have only minor gastrointestinal symptoms (bloating, constipation or diarrhea, or irritable bowel syndrome) complicates ascertainment. It is clear, however, that girls are much more often affected than boys (6). Compilation of our probands with those published (3,4,10–16) thus far show 49 of 111 (44.1%) with *ACTG2* pathogenic variants. No affected siblings with *ACTG2* pathogenic variants with parents shown not to harbor the culprit pathogenic variant have been reported. Hence, germline mosaicism remains as a possibility in such cases. Although genotype-phenotype correlations are still unclear, *de novo* pathogenic variants in *ACTG2* may convey more severe disease than when inherited (21).

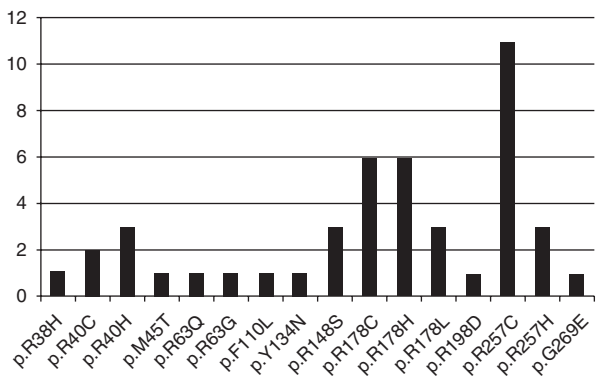

FIGURE 1. Shown are the 16 pathogenic variants reported in the *ACTG2* gene and the number of times each mutation was observed in 45 probands.

The spectrum of severity of CIPO with or without megacystis is wide, ranging from profound prenatal or neonatal megacystis with or without prune belly syndrome (22) and CIPO, to nonspecific constipation and abdominal bloating without bladder involvement. Our patients and the majority of those published with *ACTG2* pathogenic variants, have or had severe gastroparesis or bladder problems. Repeated surgical interventions have included intestinal decompression, gastrostomy, colectomy, small gut resection, ileostomy, colostomy, and Nissen fundoplication. One of our patients at 38 years of age with intestinal failure underwent transplantation of her entire intestine, stomach, liver, pancreas, and spleen.

Affected children have invariably been subject to multiple and repeated diagnostic efforts that included intestinal biopsy, radiologic studies, manometry, and cystoscopy. Now from a blood sample a precise diagnosis can be made in a week, by sequencing the *ACTG2* gene. The benefit to patients and families of early confirmation of a motility disorder not only helps avoid unnecessary intervention, but also enables institution of appropriate treatments and avoidance of secondary disorders such as malnutrition and poor growth. Moreover, knowledge of a familial *ACTG2* pathogenic variant in a parent provides an opportunity for avoidance or prevention of a recurrence via prenatal genetic diagnosis or preimplantation genetic diagnosis (23).

Acknowledgments: Whole exome sequencing of 4 families was provided by the University of Washington Center for Mendelian Genomics (UW-CMG). The bioinformatic analyses were done at The Center for Human Genetics. The authors thank Malgorzata Nowaczyk, MD and Gina Cowing, MS, CGC for referring one family.

REFERENCES

1. Berdon W, Baker D, Blanc W, et al. Megacystis-microcolon-intestinal hypoperistalsis syndrome: a new cause of intestinal obstruction in the newborn. Report of radiologic findings in five newborn girls. *AJR Am J Roentgenol* 1976;126:957–64.
2. Puri P, Shinkai M. Megacystis microcolon intestinal hypoperistalsis syndrome. *Semin Pediatr Surg* 2005;14:58–63.
3. Lehtonen HJ, Sipponen T, Tojkander S, et al. Segregation of a missense variant in enteric smooth muscle actin gamma-2 with autosomal dominant familial visceral myopathy. *Gastroenterology* 2012;143:1482–91.
4. Wangler MF, Gonzaga-Jauregui C, Gambin T, 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.
5. Amoit A, Tchikviladzé M, Joly F, et al. Frequency of mitochondrial defects in patients with chronic intestinal pseudo-obstruction. *Gastroenterology* 2009;137:101–9.
6. Gosemann JH, Puri P. Megacystis microcolon intestinal hypoperistalsis syndrome: systematic review of outcome. *Pediatr Surg Int* 2011;27: 1041–6.
7. Sabbagh C, Amiot A, Maggiori L, et al. Non-transplantation surgical approach for chronic intestinal pseudo-obstruction: analysis of 63 adult consecutive cases. *Neurogastroenterol Motil* 2013;25:680–6.
8. Ueno T, Wada M, Hoshino K, et al. A national survey of patients with intestinal motility disorder who are potential candidates for intestinal transplantation in Japan. *Transplant Proc* 2013;45:2029–31.
9. Lauro A, Zanfi C, Pellegrini S, et al. Isolated intestinal transplant for chronic intestinal pseudo-obstruction in adults: long-term outcome. *Transplant Proc* 2013;45:3351–5.
10. Halim D, Hofstra RM, Signorile L, et al. *ACTG2* variants impair actin polymerization in sporadic megacystis microcolon intestinal hypoperistalsis syndrome. *Hum Mol Genet* 2015;25:571–83.
11. Klar J, Raykova D, Gustafson E, et al. Phenotypic expansion of visceral myopathy associated with *ACTG2* tandem base substitution. *Eur J Hum Genet* 2015;23:1679–83.
12. Matera I, Rusmini M, Guo Y, 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* 2016:1–5.
13. Holla Ø, Bock G, Busk Ø, et al. Familial visceral myopathy diagnosed by exome sequencing of a patient with chronic intestinal pseudo-obstruction. *Endoscopy* 2014;46:533–7.
14. Tuzovic L, Tang S, Miller RS, et al. New insights into the genetics of fetal megacystis: *ACTG2* mutations, encoding x03B3; -2 smooth muscle actin in megacystis microcolon intestinal hypoperistalsis syndrome (Berdon syndrome). *Fetal Diagn Ther* 2015;38:296–306.
15. Thorson W, Diaz-Horta O, Foster J, et al. De novo *ACTG2* mutations cause congenital distended bladder, microcolon, and intestinal hypoperistalsis. *Hum Genet* 2013;133:737–42.
16. Annerén G, Meurling S, Olsen L. Megacystis-microcolon-intestinal hypoperistalsis syndrome(MMIHS), an autosomal recessive disorder: clinical reports and review of the literature. *Am J Med Genet* 1991;41:251–4.
17. Lu W, Xiao Y, Wang J, et al. Mutation in actin [gamma]-2 responsible for megacystis microcolon intestinal hypoperistalsis syndrome in four Chinese patients. *J Pediatr Gastroenterol Nutr* 2016;63:624–6.
18. Bonora E, Bianco F, Cordeddu L, et al. Mutations in RAD21 disrupt regulation of APOB in patients with chronic intestinal pseudo-obstruction. *Gastroenterology* 2015;148:771–82.
19. Antonucci A, Fronzoni L, Cogliandro L, et al. Chronic intestinal pseudo-obstruction. *World J Gastroenterol* 2008;14:2953–61.
20. Higman D, Peters P, Stewart M. Familial hollow visceral myopathy with varying urological manifestations. *Br J Urol* 1992;70:435–8.
21. Wangler MF, Beaudet AL. *ACTG2*-related disorders. In: Pagon RA, Adam MP, Ardinger HH, et al., eds. *GeneReviews® [Internet]*. Seattle, WA: University of Washington, Seattle; 2015.
22. Richer J, Milewicz DM, Gow R, et al. R179H mutation in ACTA2 expanding the phenotype to include prune-belly sequence and skin manifestations. *Am J Med Genet Part A* 2012;158A:664–8.
23. Milunsky A, Milunsky JM (Eds): *Genetic Disorders and the Fetus: Diagnosis, Prevention and Treatment*. Hoboken, NJ: Wiley & Sons; 2016.