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

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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

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.

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Chronic intestinal pseudo-obstruction (CIPO), the nomenclature for which has included megacystis microcolon intestinal
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-throughput genotyping 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 was 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, SAM-Tools, 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 at least one 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-methylcytosines leading to a C>T transition. Thus, the 2 amino acids, R178 and R257, are likely mutagenic insights are emerging, but further gene discovery is awaited.
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, \textit{de novo} pathogenic variants in \(ACTG2\) may convey more severe disease than when inherited (21).

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).

![Figure 1](image-url)  
**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.

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>Case 1-A</th>
<th>Case 2-A</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5-B</th>
<th>Case 6-B</th>
<th>Case 7-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>(ACTG2) Mutation</td>
<td>R257C</td>
<td>R257C</td>
<td>R257C</td>
<td>R257C</td>
<td>R40H</td>
<td>R40H*</td>
<td>R40H*</td>
</tr>
<tr>
<td>Current age</td>
<td>39</td>
<td>6</td>
<td>Prenatal</td>
<td>Died</td>
<td>11.5</td>
<td>3</td>
<td>28</td>
</tr>
<tr>
<td>Age of onset</td>
<td>Birth</td>
<td>Prenatal</td>
<td>Birth</td>
<td>Birth</td>
<td>Birth</td>
<td>Birth</td>
<td>Birth</td>
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<tr>
<td>Age of death</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>11.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Sex</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>M</td>
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<tr>
<td>Parent or sib affected</td>
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<td>–</td>
<td>–</td>
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<td>–</td>
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<td>–</td>
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<tr>
<td>Pseudo-obstruction</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Colectomy</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>+</td>
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<tr>
<td>Ileostomy</td>
<td>+</td>
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<td>–</td>
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<td>Cholecystectomy</td>
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<tr>
<td>Cholelithiasis</td>
<td>+</td>
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<td>–</td>
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<tr>
<td>Microcolon</td>
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<td>–</td>
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<td>–</td>
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<tr>
<td>Gastrostomy</td>
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<td>+</td>
<td>–</td>
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<td>–</td>
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<td>–</td>
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<tr>
<td>Nissen fundoplication</td>
<td>+</td>
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<tr>
<td>Small bowel resection</td>
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<td>+</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>GE reflux</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Cirrhosis/liver failure</td>
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<tr>
<td>Long-term TPN dependence</td>
<td>35 y</td>
<td>6 y</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Organ transplantation</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>Megacystis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Long-term bladder catheterization</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Hydronephrosis</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>Premature labor</td>
<td>+</td>
<td>–</td>
<td>N/K</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Other medical disorders</td>
<td>VSD</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>N/K</td>
<td>N/K</td>
</tr>
</tbody>
</table>

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

\*By inference.
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REFERENCES