ACTG2 Variants in Pediatric Chronic Intestinal Pseudo-obstruction With Megacystis

Jong Woo Hahn,1 Soo Young Moon,1 Min Soo Kim,1 Min Hyung Woo,1 Min Ji Sohn,1 Hyun-Young Kim,2 Moon-Woo Seong,1 Sung Sup Park,3 Sung-Hye Park,4 Jin Soo Moon,1 and Jae Sung Ko1*

Departments of 1Pediatrics, 2Surgery, 3Laboratory Medicine, and 4Pathology, Seoul National University College of Medicine, Seoul, Korea

Background/Aims
Chronic intestinal pseudo-obstruction (CIPO) is a clinically heterogeneous syndrome characterized by compromised peristalsis and intestinal obstruction. Variants of actin gamma 2 (ACTG2), a protein crucial for correct enteric muscle contraction, have been found in CIPO patients. The aim of this study is to examine the clinical features and ACTG2 variants in Korean patients with CIPO.

Methods
From January 1995 to August 2020, 12 patients diagnosed with CIPO were included and genetic analysis testing of ACTG2 was performed.

Results
Heterozygous ACTG2 missense variants were found in 6 patients (50.0%). The p.Arg257Cys variant was found in 3 patients, and p.Arg63Gln and p.Arg178His variants were found in 1 patient each. A novel variant, p.Ile193Phe, was found in 1 patient. Three patients were diagnosed at birth, 2 at the age of 1 year, and 1 at 3 years of age. Abnormal prenatal genitourinary ultrasonographic findings were found in all 6 patients; microcolon was found in 4 patients (66.7%), and megacystis in all 6 patients. The pathology showed abnormal ganglion cells as well as myopathic findings. All patients are dependent on total parenteral nutrition and are to date alive.

Conclusions
ACTG2 variants are commonly found in Korean patients with CIPO. In CIPO patients with megacystis and abnormal prenatal ultrasonography, genetic testing of ACTG2 should be considered. Molecular diagnosis of CIPO is more important than pathologic diagnosis.

(J Neurogastroenterol Motil 2022;28:104-110)

Key Words
ACTG2; Intestinal pseudo-obstruction; Megacystis; Visceral myopathy
Introduction

Chronic intestinal pseudo-obstruction (CIPO) is a disease with a wide variety of clinical and histological features, characterized by a decrease in intestinal peristalsis and symptoms of intestinal obstruction.\(^1\) A nationwide survey in the United States reported that there were 100 CIPO patients each year, and another nationwide survey in Japan reported an estimated pediatric prevalence of 3.7 per 1 million individuals.\(^2\,^3\) CIPO can involve any segment of the gastrointestinal and genitourinary tracts, and gastrointestinal symptoms, such as abdominal distention, abdominal pain and vomiting, and urinary symptoms, such as urination disorder, may occur.\(^4\,^5\) Patients with CIPO often undergo ileostomy and colostomy repeatedly.\(^4\,^6\,^7\)

CIPO is classified as either a myopathy or a neuropathy, depending on histopathology. The genetic basis of CIPO was first reported by Lehtonen et al\(^8\) in 2012. Since then, several genes related to CIPO have been discovered, of which actin gamma 2 (ACTG2) is the most common.\(^4\,^6\,^7\) The ACTG2 is located in the short arm of chromosome 2, encoding gamma 2 enteric actin, which is found in smooth muscle cells of the urinary and intestinal tracts. ACTG2 is crucial for intestinal smooth muscle contraction,\(^9\,\) and variants of ACTG2 are associated with visceral myopathy and megacystis-microcolon-intestinal hypoperistalsis syndrome (MMIHS).\(^6\,^7\,^8\) However, there are no studies on ACTG2 variants in Asia.

The purpose of this study is to investigate the clinical features and ACTG2 variants in Korean patients with CIPO.

Materials and Methods

Study Population and Data Collection

In patients with suspected intestinal obstruction, abdominal radiography is performed to determine if there is bowel dilation and air-fluid levels. If there is no fixed lesion or mechanical obstruction, it can be diagnosed as a pseudo-obstruction. If the symptoms persist for more than 6 months or postnatally for more than 2 months, CIPO may be diagnosed.\(^11\) A total of 12 patients diagnosed with CIPO at National University Hospital from January 1995 to August 2020 were included. Twelve patients were examined, and their medical records were retrospectively analyzed. Medical records included age, sex, age at onset, presenting symptoms, imaging studies, laboratory findings, and growth curves.

This study was approved by the Institutional Review Board (No. 1910-065-1070) at the performing institution. IBM SPSS Statistics 25 software was used to complete the statistical analysis. Fisher’s exact test was performed to compare the clinical features between patients with and without ACTG2 variants. A P-value < 0.05 was considered statistically significant.

Genetic Testing of ACTG2

Among the 12 patients, 11 underwent direct sequencing of ACTG2 and 1 was tested for the CIPO gene panel. The CIPO gene panel contained the following genes: ACTA2, ACTG2, CLMP, FLNA, L1CAM, LMOD1, MYH11, MYLK, POLG, RAD21, SGOL1, SOX10, and TYMP. Genomic DNA from peripheral leukocytes was extracted. All 8 coding exons and flanking introns of ACTG2 were amplified using polymerase chain reaction. Genomic DNA targeted regions were sequenced using the Sanger sequencing system. The common variants were filtered using gnomAD (http://gnomad.broadinstitute.org) and KRG (http://coda.nih.go.kr/coda/KRGDB) databases. The Human Gene Mutation Database and ClinVar were used to search for known pathogenic variants. The sequence variants were evaluated by computational (in silico) predictive programs using PolyPhen-2, SIFT, and MutationTaster. The effect of sequence variants was determined at the nucleotide and amino acid levels, and the potential impact of the variants on the protein could be seen. In addition, the pathogenicity of sequence variants was evaluated using the 2015 American College of Medical Genetics ad Genomics (ACMG) guidelines.\(^12\)

| DNA change | Amino acid change | Variant type | Allele frequency | ACMG classification |
|------------|------------------|--------------|-----------------|---------------------|
| 769C>T     | p.Arg257Cys      | Missense     | 3/6 (50.0%)     | PM1, PM2, PP2, PP3, PP5 Likely pathogenic |
| 188G>A     | p.Arg63Gln       | Missense     | 1/6 (16.7%)     | PM1, PM2, PP2, PP3  Likely pathogenic |
| 577A>T     | p.Ile193Phe      | Missense     | 1/6 (16.7%)     | PM1, PM2, PP2, PP3  Likely pathogenic |
| 533G>A     | p.Arg178His      | Missense     | 1/6 (16.7%)     | PM1, PM2, PP2, PP3, PP5 Likely pathogenic |

ACTG2, actin gamma 2; CIPO, chronic intestinal pseudo-obstruction; ACMG, American College of Medical Genetics and Genomics; PM, pathogenic moderate; PP, pathogenic supporting. |
Results

ACTG2 Variant Analysis

Twelve patients were analyzed, and 6 were identified with ACTG2 variants. All 6 cases were sporadic and without a family history. All variants were heterozygous missense variants, of which 5 were arginine substitutions. The variants c.769C>T, p.Arg257Cys (50.0%) was found in 3 patients; c.188G>A, p.Arg63Gln (16.7%) in 1 patient; c.533G>A, p.Arg178His (16.7%) in 1 patient; and c.577A>T, p.Ile193Phe (16.7%) in 1 patient (Table 1). p.Arg257Cys, p.Arg178His, and p.Arg63Gln are previously reported variants and p.Arg257Cys is the most common. p.Ile193Phe is a novel variant that is not observed among the normal population and is located at highly conserved loci in various species. All variants were considered likely pathogenic by the ACMG classification.

Comparison of Clinical Features Between Patients With and Without ACTG2 Variants

Table 2 compares patients with and without ACTG2 variants. The presence of megacystis and abnormal prenatal ultrasonography showed a statistically significant difference between the 2 groups, but others, including age of diagnosis, microcolon, malrotation, hydronephrosis, and neurogenic bladder, were not statistically significant.

Clinical Features of Patients With ACTG2 Variants

The clinical characteristics of the 6 patients with ACTG2 variants are summarized in Table 3. Four patients were female, and 2 were male. Their current ages range from 44 months to 27 years with a median value of 8.5 years. Three patients were diagnosed at birth: 2 at age 1 year, and 1 at age 3 years, with the median age at diagnosis being 7 months. The initial clinical symptoms were constipation in 5 patients, abdominal distention in 3, and vomiting in 2. On prenatal ultrasonography, all patients showed abnormal genitourinary findings, such as enlarged bladder, ovarian cyst, and hydronephrosis. Megacystis was observed in all 6 patients. Microcolon was found in 4 patients (66.7%), and they were diagnosed with MMIHS. Malrotation was observed in 3 patients (50.0%), hydronephrosis in 4 (66.7%), and neurogenic bladder in 2 (33.3%). Pathological studies in all patients showed abnormalities of the muscle layer and ganglion cells. Thinning of the muscle layer and vacular changes of the muscle layer were found in 3 patients each. Nuclear palisading and acidophilic globular bodies were found in 1 patient each (Figure). All patients had hypoganglionosis and immature ganglion cells, and ganglion cell shapes were smaller than normal.

Long-term Outcomes in Patients With ACTG2 Variants

The follow-up period of patients ranged from 44 months to 24 years with a median value of 62 months. All patients underwent abdominal surgeries. Jejunostomy was performed in 2 patients, ileostomy in 6 patients, and colostomy in 1 patient. Cholecystectomy, gastrectomy, and colectomy were performed in Case 3. None of the patients received an intestinal transplant and all patients are still alive. Long-term CIC was performed in 2 patients (33.3%), and Case 6 underwent vesicotomy 5 months immediately after diagnosis. All patients remain dependent on PN with oral feeding. Among them, Case 4 recently discontinued PN, whereas Case 5 recently initiated PN. Central line-associated bloodstream infection (CLABSI) was observed in 3 patients, and fatty liver was observed in 2 patients. One patient suffered recurrent small intestinal bacterial overgrowth and currently takes cyclic antibiotics. Pyridostigmine, an acetylcholinesterase inhibitor that stimulates gastrointestinal motility, was administered to 3 patients, and symptoms improved in 2 of them.

Discussion

In this study, we investigated the spectrum of clinical features and ACTG2 variants in Korean patients with CIPO. Among the 12 CIPO patients, ACTG2 variants were found in 6 patients.
| Clinical data                  | Case 1                        | Case 2                        | Case 3                        | Case 4                        | Case 5                        | Case 6                        |
|-------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
| **ACTG2 mutation**            | c.769C>T: p.Arg257Cys         | c.188G>A: p.Arg63Gln         | c.577A>T: p.Ile193Phe        | c.769C>T: p.Arg257Cys        | c.769C>T: p.Arg257Cys        | c.533G>A: p.Arg178His         |
| Current age                   | 15 yr                        | 4 yr                         | 27 yr                        | 12 yr                        | 44 mo                        | 5 yr                         |
| Age at onset                  | 14 mo                        | Birth                        | 3 yr                         | 15 mo                        | Birth                        | Birth                        |
| Sex                           | F                            | F                            | F                            | M                            | M                            | M                            |
| Prenatal US                   | Enlarged bladder             | Enlarged bladder             | Ovarian cyst                 | Ovarian cyst                 | Ovarian cyst                 | Megacystis                   |
|                               | Hydronephrosis               |                              |                              |                              |                              | Hydronephrosis               |
| Presenting symptom            | Constipation                 | Bowel distention             | Vomiting                     | Abdomen distention           | Constipation                 | Constipation                 |
|                               |                              |                              | Constipation                 |                              |                              |                              |
| Microcolon                    | +                            | +                            | –                            | +                            |                              |                              |
| Malrotation                   | +                            | +                            | –                            | +                            |                              |                              |
| Surgery                       | Ileostomy                    | Jejunostomy                  | Ileostomy                    | ileostomy                    | ileostomy                    | ileostomy                    |
| Colostomy                     | Ileostomy                    | Ileostomy                    | Cholecystectomy              | Gastrectomy                  | Colectomy                    |                              |
| Megacystis                    | +                            | +                            | +                            | +                            | +                            | +                            |
| Hydronephrosis                | +                            | +                            | –                            | –                            | –                            | +                            |
| Neurogenic bladder            | –                            | –                            | –                            | +                            | +                            | –                            |
| Long-term CIC                 | –                            | –                            | –                            | +                            | +                            | +                            |
| Long-term home PN             | +                            | +                            | +                            | +                            | –                            | +                            |
| CLABSI                        | +                            | +                            | –                            | –                            | –                            | +                            |
| Fatty liver                   | +                            | –                            | –                            | +                            | –                            | –                            |
| Pathology                     | Nuclear palisading in the    | Vacuolar change in the       | Acidophilic globular         | Thinning in the inner and    | Marked thinning, and         | Marked thinning, and         |
|                               | inner muscle layer           | inner muscle layer           | bodies in the outer muscle   | outer muscle layer           | vacuolation in the outer      | vacuolation in the inner      |
|                               |                               |                              | layer                        |                              | muscle layer                 | muscle layer                 |

*ACTG2*, actin gamma 2 gene; F, female; M, male; US, United States; CIC, clean intermittent catheterization; PN, parenteral nutrition; CLABSI, Central Line Associated Blood Stream Infection; +, feature present; –, feature absent.
(50.0%), with p.Arg257Cys being the most common. All cases were sporadic, and we reported a novel ACTG2 variant. All patients with ACTG2 variants showed megacystis, abnormal prenatal ultrasonography, and had pathological abnormalities in the muscle layer and ganglion cells. All patients were dependent on PN and are alive to date. We showed the indication and importance of ACTG2 genetic testing in patients with CIPO.

In this study, 4 ACTG2 variants were identified in 6 children, and 3 of the 4 variants had arginine substitutions. In previous studies, 44.0-62.0% of CIPO patients were positive for ACTG2 variants, and arginine substitutions accounted for 49.0% of total variants. All variants in this study are heterozygous missense variants, which is consistent with findings of other studies. p.Arg257Cys, p.Arg63Gln, and p.Arg178His have been previously reported and are recurrent pathogenic variants, while p.Ile193Phe is novel. All variants including a novel variant were considered likely pathogenic by the ACMG classification. These known missense variants are reported to affect protein function, impair ACTG2 polymerization, and reduce smooth muscle cell contractility. Previous studies have shown that arginine substitution at several different sites affects the function of actin filament, contributing to poor contractility of smooth muscle in COS7, CRL-1776 and U2OS cells. In this study, p.Arg257Cys was the most common variant, and p.Arg178Cys and p.Arg257Cys were mutation hot spots in other studies. All patients in this study were sporadic, and these variants may have occurred de novo. Cases 1, 4, and 5 had the same variant as p.Arg257Cys, but the clinical features were different. Cases 1 and 4 were diagnosed with MMIHS. However, Case 5 did not have MMIHS because microcolon was not present. In previous studies, microcolon was reported in 5 out of 20 (25.0%) patients with p.Arg257Cys. These findings suggest that visceral myopathy and MMIHS are one entity with a continuum of various symptoms. Some studies have shown a genotype-phenotype correlation. Arginine missense substitutions in ACTG2 had a 63.8% chance of requiring intestinal transplant, total PN dependence, or disease-related mortality. In this study, all patients were dependent on total PN, regardless of arginine substitutions. In addition, all variants in this study may occur de novo, which may present with a more severe phenotype than when inherited. All patients in this study showed a severe phenotype, and the number of patients was small; therefore, it was difficult to evaluate a genotype-phenotype correlation. Also, there was no racial difference between Asian and Caucasian patients in ACTG2 variants, because p.Arg257Cys, frequently found in Caucasians, was the most common in this study. Out of 12 patients, 1 patient was examined with the CIPO gene panel and an ACTG2 variant was identified. We found that in the patient group with ACTG2 variants, there were 4 female and 2 male patients, which is consistent with previous studies showing a female preponderance.
100.0% of patients with ACTG2 variants had megacystis. On prenatal ultrasonography, all patients with ACTG2 variants had genitourinary abnormalities. All MMIHS patients with ACTG2 variants had prenatally evident fetal megacysts.Taken together, if patients with CIPO have genitourinary abnormalities on prenatal ultrasonography or presence of megacystis, the evaluation of visceral myopathy with ACTG2 variants should be considered.

Thinning and vacuolar changes of the muscle layer found in this study are key features of visceral myopathy. Nuclear palisading is a nonspecific histopathologic finding of myopathy, which is occasionally observed in other studies. Acidophilic globular body is a novel and pathologic finding, which is not observed in normal intestinal muscle proper, and appears to be related with muscular degenerative changes. Also, ganglion cell abnormalities such as hypoganglionosis and immature ganglia were found in all 6 patients with ACTG2 variants. In previous studies, ganglion cell abnormalities were observed in patients with visceral myopathy or MMIHS. Ganglion cell abnormalities may be secondary changes in myopathy due to ACTG2 variants. Because hypoganglionosis or dysmorphic ganglion cells may be seen in CIPO with ACTG2 variants, it is difficult to diagnose by histopathology alone, and molecular diagnosis is more accurate than histologic diagnosis.

In this study, different abdominal surgeries were performed. Six patients received 26 surgeries and a mean of 4.3 surgeries per patient, with the most common operation being ostomy, which is similar to the results of other studies. In this study, all patients were dependent on PN, which is similar to the results of other studies. Long-term home PN causes several complications. CLABS was observed in 3 patients (50.0%), and fatty liver was observed in 2 patients (33.3%) in this study. To reduce the complications of long-term PN, fish oil-based lipid emulsions are used and enteral feeding is maintained. Thus, cirrhosis may not develop in this study. Previous studies have reported that the survival rate was 12.6-19.7%, and the oldest survivor was 25 years old. Mortality was associated with a short small intestine, involvement of the urinary system, < 1 year of age of onset, and myopathy on histology. In this study, the survival rate of CIPO was 100.0%, and the oldest survivor was 27 years old. The remarkable improvement in prognosis in this study may have resulted from improvements in nutritional management and supportive care with intestinal rehabilitation programs. In this study, pyridostigmine was administered to 3 patients, and was effective in 2 of them. Pyridostigmine has been demonstrated to be effective in colonic decompression in pediatric CIPO patients.

The limitation of this study is that the number of CIPO patients was small, and the study was conducted at a single center. A multicenter study involving a large number of Korean patients with CIPO is needed.

In conclusion, ACTG2 variants are commonly found among CIPO patients in Korea. In CIPO patients with megacystis and abnormal prenatal ultrasonography, genetic testing of ACTG2 should be considered. Molecular diagnosis of CIPO is important because it is difficult to differentiate myopathy from neuropathy by pathological findings alone. ACTG2 genetic analysis can help avoid invasive diagnostic interventions such as full-thickness bowel biopsy.

Financial support: This study was supported by grant (No. 0420203090) from Seoul National University Hospital.

Conflicts of interest: None.

Author contributions: Jong Woo Hahn: collecting, interpreting data, and drafting the manuscript; Soo Young Moon, Min Soo Kim, Min Hyung Woo, and Min Ji Sohn: interpreting data; Hyun-Young Kim: conducting the study; Moon-Woo Seong, Sung Sup Park, and Sung-Hye Park: conducting the study and interpreting data; Jin Soo Moon: conducting the study, interpreting the data, and revising the manuscript; and Jae Sung Ko: planning and conducting the study, interpreting the data, and revising the manuscript.

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