



ACTG2 Variants in Pediatric Chronic Intestinal Pseudo-obstruction With Megacystis

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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.

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Key Words

ACTG2; Intestinal pseudo-obstruction; Megacystis; Visceral myopathy

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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.^{1,4} Patients with CIPO often undergo ileostomy and colostomy repeatedly.⁴⁻⁷

CIPO is classified as either a myopathy or a neuropathy, depending on histopathology. The genetic basis of CIPO was first reported by Lehtonen et al⁸ in 2012. Since then, several genes related to CIPO have been discovered, of which actin gamma 2 (*ACTG2*) is the most common.^{4,6,7,9} The *ACTG2* is located in the short arm of chromosome 2, encoding gamma 2 enteric actin, which is found in the smooth muscle cells of the urinary and intestinal tracts. *ACTG2* is crucial for intestinal smooth muscle contraction,^{9,10} and variants of *ACTG2* are associated with visceral myopathy and megacystis-microcolon-intestinal hypoperistalsis syndrome (MMIHS).^{6,7,10,11} 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 per-

sist 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 and Genomics (ACMG) guidelines.¹²

Table 1. Variants of *ACTG2* Gene Among Patients With Chronic Intestinal Pseudo-obstruction

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.¹²

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, neurogenic bladder, long-term clean intermittent catheterization (CIC), and long-term home parenteral nutrition (PN), were not statistically significant.

Clinical Features of Patients With *ACTG2* Variants

The clinical characteristics of the 6 patients with *ACTG2* vari-

Table 2. Comparison of Clinical Feature Between Patients With and Without *ACTG2* Mutations

Clinical feature	<i>ACTG2</i> (+) (n = 6)	<i>ACTG2</i> (-) (n = 6)	<i>P</i> -value
Age at diagnosis	7 months	Birth	> 0.05
Prenatal ultrasonography	6/6 (100%)	1/6 (16.7%)	0.015
Microcolon	4/6 (66.7%)	0/6 (0.0%)	0.061
Malrotation	3/6 (50%)	0/6 (0.0%)	> 0.05
Megacystis	6/6 (100%)	0/6 (0.0%)	0.002
Hydronephrosis	4/6 (66.7%)	1/6 (16.7%)	> 0.05
Neurogenic bladder	2/6 (33.3%)	0/6 (0.0%)	> 0.05
Long-term CIC	2/6 (33.3%)	0/6 (0.0%)	> 0.05
Long-term home PN	6/6 (100%)	6/6 (100.0%)	> 0.05

CIC, clean intermittent catheterization; PN, parenteral nutrition.

ants 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 vacuolar 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 all 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

Table 3. Clinical Features and Natural History of Chronic Intestinal Pseudo-obstruction With ACTG2 Variants

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	F	M	M
Prenatal US	Enlarged bladder	Enlarged bladder Hydronephrosis	Ovarian cyst	Ovarian cyst	Megacystis	Hydronephrosis
Presenting symptom	Constipation	Bowel distention	Vomiting Constipation	Abdomen distention Constipation	Pseudo-obstruction Constipation	Bowel obstruction Vomiting Constipation Bowel distention
Microcolon	+	+	-	+	-	+
Malrotation	+	+	-	-	-	+
Surgery	Ileostomy Colostomy	Jejunostomy Ileostomy	Ileostomy Cholecystectomy Gastrectomy Colectomy	Ileostomy	Ileostomy	Jejunostomy Ileostomy
Megacystis	+	+	+	+	+	+
Hydronephrosis	+	+	+	-	-	+
Neurogenic bladder	-	-	-	+	+	-
Long-term CIC	-	-	-	+	+	Vesicotomy (2014.12-2015.05)
Long-term home PN	+	+	+	+	-	+
CL/ABSI	+	+	-	-	-	+
Fatty liver	+	-	-	+	-	-
Pathology	Nuclear palisading in the inner muscle layer	Vacuolar change in the inner muscle layer	Acidophilic globular bodies in the outer muscle layer	Thinning in the inner and outer muscle layer	Marked thinning, and vacuolation in the outer muscle layer	Marked thinning, and vacuolation in the inner muscle layer

ACTG2, actin gamma 2 gene; F, female; M, male; US, United States; CIC, clean intermittent catheterization; PN, parenteral nutrition; CL/ABSI, Central Line Associated Blood Stream Infection; +, feature present; -, feature absent.

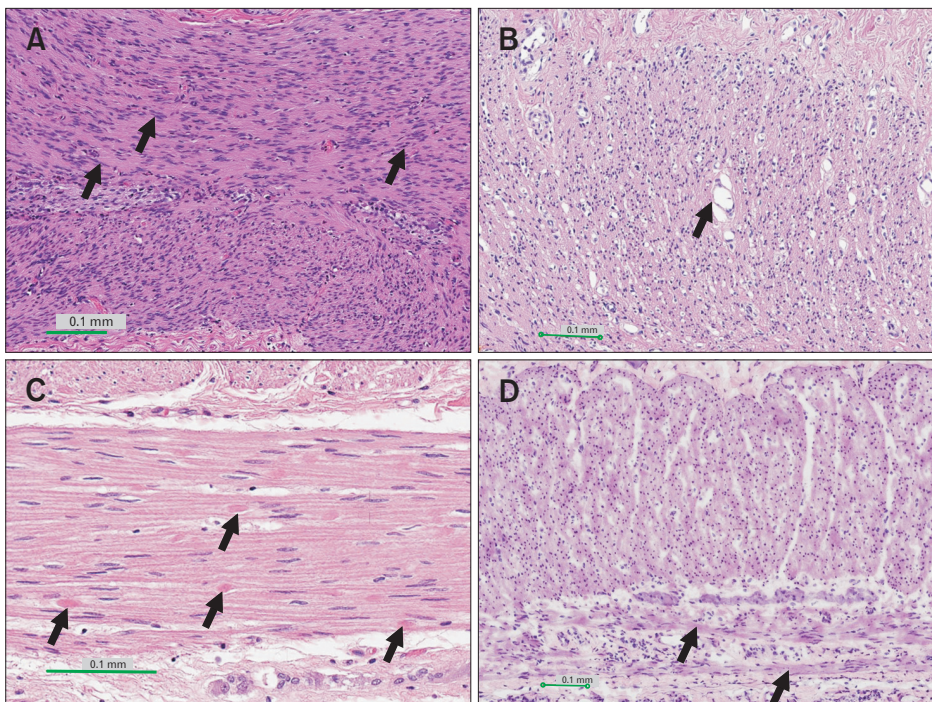


Figure. Pathologic findings of patients with *ACTG2* variants. (A) [Case 1] The inner circular muscle layer shows nuclear palisading (arrows). (B) [Case 2] The inner circular muscle layer shows vacuolar change (arrow). (C) [Case 3] The outer layer of the muscle shows acidophilic globular bodies (arrows). (D) [Case 5] The outer muscle layer shows marked thinning (arrows) (H&E, bar = 0.1 mm).

(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.^{13,14} All variants in this study are heterozygous missense variants, which is consistent with findings of other studies.¹⁵ p.Arg257Cys, p.Arg63Gln, and p.Arg178His have been previously reported and are recurrent pathogenic variants,^{7,10} 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.¹⁵ 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.^{8,9} In this study, p.Arg257Cys was the most common variant, and p.Arg178Cys and p.Arg257Cys were mutation hot spots in other studies.^{6,10,13} 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.¹³ 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.¹³ 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.⁶ 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.^{6,7,10} 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.^{13,16} In this study, megacystis was found in all 6 patients. In previous studies, 85.0-

100.0% of patients with *ACTG2* variants had megacystis.^{6,13} On prenatal ultrasonography, all patients with *ACTG2* variants had genitourinary abnormalities. All MMIHS patients with *ACTG2* variants had prenatally evident fetal megacystis.¹⁷ 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 findings of myopathy, which is occasionally observed in other studies.^{18,19} 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.^{2,10,17,20-24} Ganglion cell abnormalities may be secondary changes in myopathy due to *ACTG2* variants.¹⁰ 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.^{7,13,22,25} In this study, all patients were dependent on PN, which is similar to the results of other studies.^{13,25,26} Long-term home PN causes several complications.^{27,28} CLABSI 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.^{11,16} 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.^{7,16,22} Mortality was associated with a short small intestine, involvement of the urinary system, < 1 year of age of onset, and myopathy on histology.²⁹ 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.^{7,26,30,31} 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.^{32,33}

The limitation of this study is that the number of CIPO pa-

tients 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.

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Conflicts of interest: None.

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