HHS Public Access

Author manuscript

Am J Med Genet A. Author manuscript; available in PMC 2017 September 08.

Published in final edited form as:

Am J Med Genet A. 2016 November ; 170(11): 2965–2974. doi:10.1002/ajmg.a.37857.

Visceral Myopathy: Clinical and Molecular Survey of a Cohort of Seven New Patients and State of the Art of Overlapping Phenotypes

Carolina Araujo Moreno[1], **Konradin Metze**[2], **Elizete Aparecida Lomazi**[3], **Débora Romeo Bertola**[4], **Ricardo Henrique Almeida Barbosa**[5], **Viviana Cosentino**[6], **Nara Sobreira**[7], and **Denise Pontes Cavalcanti**[1,*]

[1]Faculty of Medical Sciences, Departmentof Medical Genetics, State University of Campinas, Campinas, Brazil [2]Faculty of Medical Sciences, Department of Pathology, State University of Campinas, Campinas, Brazil [3]Faculty of Medical Sciences, Department of Pediatrics, State University of Campinas, Campinas, Brazil [4]Genetic Unit, Faculty of Medicine, Children's Institute, University of São Paulo, São Paulo, Brazil [5]Department of Morphology, Medical Genetics Center, Federal University of São Paulo, São Paulo, Brazil [6]CEMIC (Center for Medical Education and Clinical Research), Buenos Aires, Argentina [7]Department of Pediatrics, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland

Abstract

Visceral motility dysfunction is a key feature of genetic disorders such as megacystis-microcolon-intestinal hypoperistalsis syndrome (MMIHS, MIM moved from 249210 to 155310), chronic intestinal pseudo-obstruction (CIPO, MIM609629), and multisystemic smooth muscle dysfunction syndrome (MSMDS, MIM613834). The genetic bases of these conditions recently begun to be clarified with the identification of pathogenic variants in *ACTG2*, *ACTA2*, and *MYH11* in individuals with visceral motility dysfunction. The MMIHS was associated with the heterozygous variant in *ACTG2* and homozygous variant in *MYH11*, while the heterozygous variant in *ACTA2* was observed in patients with MSMDS. In this study, we describe the clinical data as well as the molecular investigation of seven individuals with visceral myopathy phenotypes. Five patients presented with MMIHS, including two siblings from consanguineous parents, one had CIPO, and the other had MSMDS. In three individuals with MMIHS and in one with CIPO we identified heterozygous variant in *ACTG2*, one being a novel variant (c.584C>T—p.Thr195Ile). In the individual with MSMDS we identified a heterozygous variant in *ACTA2.* We performed the whole-exome sequencing in one sibling with MMIHS and her parents; however, the pathogenic variant responsible for her phenotype could not be identified. These results reinforce the clinical and genetic heterogeneity of the visceral myopathies. Although many cases of MMIHS are

*Correspondence to: Denise Pontes Cavalcanti, Departamento de Genética Médica, FCM, Unicamp. R. Tessália Vieira de Camargo, 126, 13083-887, Campinas, São Paulo, Brazil, denisepcavalcanti@gmail.com.

Supporting Information: Additional supporting information may be found in the online version of this article at the publisher's web-site.

Conflict of interest: The authors have no conflict of interest to declare.

associated with *ACTG2* variants, we suggest that other genes, besides *MYH11*, could cause the MMIHS with autosomal recessive pattern.

Keywords

visceral myopathy; *ACTG2*; *ACTA2*; *MYH11*

Introduction

Visceral motility dysfunction is characterized by symptoms related to dysmotility of gastrointestinal and urinary tract, a key feature of genetic disorders such as megacystis-microcolon-intestinal hypoperistalsis syndrome (MMIHS, MIM moved from 249210 to MIM155310); chronic intestinal pseudo-obstruction (CIPO, MIM15531); and multisystemic smooth muscle dysfunction syndrome (MSMDS, MIM613834). There is a significant overlap among these phenotypes and the recent advances regarding the genetic bases of these conditions contributed to improve the knowledge.

MMIHS is a severe condition characterized by functional obstruction in the urinary and gastrointestinal tract resulting in marked dilatation of the bladder, underdevelopment of the colon, and decreased or absent intestinal peristalsis [Gosemann and Puri, 2011]. More than 200 described cases are sporadic, and the recent identification of de novo heterozygous variant in *ACTG2* confirmed the autosomal dominant (AD) pattern in many, but not in all [Thorson et al., 2014; Wangler et al., 2014]. The occurrence of MMIHS in the offspring of consanguineous parents and/or recurrence in siblings with asymptomatic parents lent support to the hypothesis of autosomal recessive (AR) inheritance [Mc Laughlin and Puri, 2013]. In addition, more recently the AR pattern was demonstrated by Gauthier et al. [2014], who reported a homozygous loss-of-function variant in *MYH11* in an individual with MMIHS born to consanguineous parents.

ACTG2 is associated with a less severe phenotype of visceral myopathy named chronic intestinal pseudo-obstruction (CIPO) in familial cases with AD inheritance [Lehtonen et al., 2012; Holla et al., 2014; Klar et al., 2015]. Currently, MMIHS and CIPO are understood as part of a phenotypic spectrum related to pathogenic variants in *ACTG2* [Wangler et al., 2014]. The MSMDS is a severe condition with significant overlap with MMIHS, but with congenital mydriasis and vascular abnormalities. This phenotype has been associated with de novo heterozygous variant in the codon 179 in *ACTA2* [Milewicz et al., 2010].

Here, we report the clinical and molecular investigation of seven individuals with visceral smooth muscle diseases showing pathogenic variants in *ACTG2* (four individuals) and *ACTA2* (one individual) in sporadic cases, and two siblings with MMIHS without pathogenic variant identified. In addition, we present the state of the art of molecular bases of visceral myopathy based on a review of the related disorders.

Materials and Methods

The present study was approved by National Committee of Ethics in Research (CONEP). For all the participants and families the informed consent was duly obtained.

Patients

Seven individuals with visceral myopathy were included: Five individuals presenting typical findings of MMIHS (patients 1, 2, 3, 4, and 5), a sixth individual with CIPO phenotype (patient 6), and one with clinical features suggestive of MSMDS (patient 7)— Table I. The parents were included in the study when their DNA was available. Clinical data, including family history, and clinical follow up, were obtained from all patients.

Molecular Study

The investigation strategy was defined based on the phenotype. The sporadic cases of MMIHS and CIPO were studied by Sanger sequencing of *ACTG2*. The molecular investigation of the family with two affected siblings with MMIHS was performed only in one patient (patient 2), because DNA from her brother was not available. When the study was designed, *ACTG2* was the only gene associated with MMIHS. Despite the presence of parental consanguinity and recurrence in siblings suggesting an AR inheritance in this family, we initially tested for dominant loci based on the hypothesis of parental gonadal mosaicism. Due to overlap between the MMIHS and MSMDS phenotype, we also performed Sanger sequencing of *ACTA2* in patient 2; however, since the sequencing was negative for both genes, the whole-exome sequencing (WES) was performed for the trio (patient 2 and her parents).

In the patient with signs of MSMDS, *ACTG2*, and *ACTA2* were sequenced by the Sanger method. At first, we tested *ACTG2* because there was no information about vascular abnormality in the patient at that time. This data were provided during the follow up.

The molecular tests were performed on DNA extracted from lymphocytes (patients 2, 3, 4, 5, and 7, parents of patients 1, 2, 3, 4, and 5, and mother of patient 7); and from saliva (patient 6 and her mother). DNA sample from patient 1, and the fathers of patients 6 and 7 were unavailable.

For the *ACTG2* and *ACTA2* Sanger sequencing, the primers were designed to encompass all coding exons and their flanking regions. The gene nucleotide numbering of *ACTG2* and *ACTA2* is according to the sequence NM_001615 and NM_001613, respectively. The primers and the details about Sanger sequencing of *ACTG2* and *ACTA2* are described in the supplementary material (SM1). For the WES, the Agilent SureSelect Human All Exon V4–51Mb kit (Agilent Technologies, Santa Clara, CA) was used to capture the target regions. The WES (paired end 100 bp reads) was performed using the Illumina HiSeq2500 platform (Illumina, Inc. San Diego, CA). The phenoDB analysis tool was applied to filter and prioritize rare functional variants (missense, nonsense, splice site variants, and indels) [Sobreira et al., 2015]. The detailed information regarding the WES is described in the supplementary material (SM2). For the novel variants, the pathogenicity was tested in different ways: (i) in silico analysis by PolyPhen-2, Mutation Taster, MutPred, Panther,

SNAP, Phd-SNP, and SNPs&GO (web sources); (ii) research in different databases: dbSNP, 1000 Genomes project data, and Exome Variant Server (web sources); (iii) sequencing of the parental samples whenever possible and 100 alleles from control individuals. We used the HOPE program to analyze the structural and functional effects of novel variants [Venselaar et al., 2010].

Review of the Literature

Due to similarities between the MMIHS and MSMDS, and the recent advances in understanding their genetic bases, we reviewed both phenotypes in order to better characterize these diseases, in an attempt to verify a possible genotype–phenotype correlation. We performed the literature review through the PubMed site using the following key words: "megacystis microcolon intestinal hypoperistalsis," "multisystemic smooth muscle dysfunction," "*ACTG2*," "*ACTA2*," and "*MYH11*." Only the articles reporting patients with molecular diagnosis were included. For the cases reported more than once we included the first description.

Results

Clinical Description

The clinical data and molecular results of the individuals are summarized in Table I. In addition, more detailed clinical information from each patient is presented below according to the respective phenotypes. In all cases reported here, the parents were asymptomatic.

Megacystis-Microcolon-Intestinal Hypoperistalsis Syndrome (MMIHS)

Patients 1 and 2 (family 1)—A male and a female infant, respectively, were born from consanguineous parents (F = 0.0625) and both manifested the first symptoms during the prenatal period. Hydronephrosis and megacystis were identified in both by the 13 and 20th week, respectively. While severe oligohydramnios was observed at19 weeks in patient 1, polyhydramnios was detected after 25th week in patient 2. Patient 1 was unsuccessfully treated with a vesicoamniotic shunt. After his birth, symptoms of gastrointestinal hypoperistalsis (bilious emesis, enteral feeding intolerance, and absent meconium elimination), and microcolon were observed suggesting MMIHS. He died due to renal failure at the end of the 1st month. The presence of mydriasis was not evaluated. The diagnosis of MMIHS in patient 2 was considered during the pregnancy because of megacystis plus the previously affected brother. After birth, severe gastrointestinal hypoperistalsis and microcolon were confirmed. In addition, she presented with congenital mild mydriasis with pupils slightly reactive to the light reflex. She was followed by us up to 6 months, when she transferred to another country in order to try the multivisceral transplantation, which finally occurred when she was 16 months old. However, she died due to an infection 4 months after the transplantation.

Patient 3 (family 2)—A male infant and the first child of young parents. The megacystis was identified at the 21st week of pregnancy. Hyperechogenic kidneys, hydroureter, club feet, and oligohydramnios were seen on the subsequent prenatal ultrasound examination. After birth, the patient developed symptoms of gastrointestinal hypomotility such as enteral

feeding intolerance, abdominal distention, and absence of meconium elimination. Microcolon was detected and MMIHS was diagnosed. There was no evidence of mydriasis. Despite the supportive therapy he died at 7 months.

Patient 4 (family 3)—A female infant and the second child of young parents. Megacystis and polyhydramnios were detected by prenatal ultrasound at the 30th week of pregnancy. After birth, she presented with mild gastrointestinal hypomotility symptoms and tolerated oral feeding during 4 months. After this period, she developed progressive symptoms of intestinal dysmotility that included enteral feeding intolerance and chronic constipation without evidence of mechanical obstruction. She required parental nutrition and despite the supportive therapy, she died at 11 months due to sepsis. There was no evidence of mydriasis.

Patient 5 (family 4)—A male infant and the second child of young parents. Megacystis was identified by prenatal ultrasound at the 35th week. The amniotic fluid volume was normal. After birth, intestinal hypomotility symptoms such as vomiting, constipation, and abdominal distention and microlon were identified, supporting the diagnosis of MMIHS. There was no evidence of mydriasis. He did not tolerate oral feeding and is total parental nutrition– nutrition (TPN)-dependent since the first days of his life. Due to meningitis, the patient developed secondary hydrocephalus.

Patients 1, 2, and 3 presented with signs of prune-belly at birth. All patients were parental nutrition-dependent, but the patient 4 tolerated oral feeding for a short period.

Chronic Intestinal Pseudo-Obstruction (CIPO)

Patient 6 (family 5): A female child with two asymptomatic older brothers. Although her mother reported that bladder dilatation was observed during a prenatal ultrasound examination in the 3rd trimester, at birth there was no evidence of megacystis or visceral motility dysfunction. The symptoms of CIPO, such as recurrent episodes of vomiting, constipation, and abdominal distention, began when she was 2 years old. These acute symptoms occurred during a varicella zoster infection and were associated with urinary retention, and transitory megacystis during some episodes. The urinary symptoms spontaneously resolved after the improvement of the gastrointestinal complaints. Besides episodic megacystis, no other abnormality was identified in the urinary system. During the acute episodes of intestinal pseudo-obstruction, the treatment was conservative wherever possible and consisted of oral fasting, tube for gastrointestinal decompression, and parenteral nutrition with gradual transition to enteral feeding according to her clinical recovery. So far, her nutrition is totally enteral. Therapeutic surgical procedures (Table I) were performed only when conservative measures were inefficient. Only the mother was available for molecular study.

Multisystemic Smooth Muscle Dysfunction Syndrome (MSMDS)

Patient 7 (family 6)—She is the first child of young parents. During the pregnancy, megacystis, hydroureter, and hydronephrosis were identified by the ultrasound examination. After birth she presented with congenital mydriasis, intestinal malrotation, and symptoms of intestinal hypoperistalsis including vomiting and constipation. Cardiovascular abnormalities

included atrial septal defect, ductus arteriosus aneurysm, and pulmonic valve dilatation. Surgical closure of the ductus arteriosus was performed in the 1st month of life. MRI/MRA performed at 10 months showed increased T2 and FLAIR signal intensity in the supratentorial region, predominantly in the centrum semiovale, corona radiate, and frontal subcortical region suggestive of terminal ischemic injuries. Bilateral stenosis of the extracranial carotid artery with abnormally straightened arterial course was noted, including the terminal segments and hypoplastic posterior circulation system with significant reduction of basilar flow. The posterior circulation has been maintained through the communicating system. There is no evidence of aneurysm. She was partially fed by parenteral nutrition in her first months of life and currently she receives an enteral diet by gastrostomy. Only her motherwas available for molecular study.

Molecular Study

As shown in Table I, we found four heterozygous pathogenic variants in *ACTG2* (patients 3, 4, and 5 with MMIHS and patient 6 with CIPO), and one heterozygous variant in *ACTA2* in patient 7 with MSMDS. The DNA chromatograms with the variants are shown in the supplementary material (Fig.—SM3). In *ACTG2*, the variants c.532C>T, c.770G>A, and c. 533G>T were identified, respectively, in patients 3, 4, and 5. They were absent in the parents and considered apparently de novo variants. The change in the same gene in patient 6 (c. 584C>T) is a novel mutation. It was not detected in her mother and the DNA from her father was not available. This variant was predicted as pathogenic by all software programs except by SNAP and SNPs&GO (see pathogenicity prediction in the supplementary material—SM4). According to the HOPE program, the substitution of threonine by isoleucine at the position 195 is probably damaging to the protein, because unlike the wild-type, this change introduces a larger molecular weight and more hydrophobic residue in the mutated protein, possibly affecting the hydrogen bond formation between the original residue and the methionine at position 191. In addition, the mutant residue is located in the surface of the protein, near a highly conserved position probably disturbing interactions with other molecules or other parts of the protein. This change was absent in 100 alleles from control individuals. Patient 7 presented with the known c.535C>T variant in *ACTA2*. It was not detected in her mother and the DNA from her father was unavailable.

The molecular investigation of the family with two affected sibs with MMIHS (patients 1 and 2), was performed only in patient 2. The *ACTA2* and *ACTG2* sequencing did not show a pathogenic variant. In the WES analysis, we initially prioritized the identification of homozygous variants. We also considered other possibilities such as AR—compound heterozygous and AD with recurrence explained by parental gonadal mosaicism. A total of 23 candidate genes were identified: AR—homozygous (10 genes), AR— compound heterozygous (four genes), and AD (nine genes), but no association between a specific gene and the phenotype has yet been proven for the candidates genes. We did not identified pathogenic variant in *MYH11* in patient 2.

Review of the Literature

Sixteen articles including clinical and molecular data from individuals with MMIHS or MSMDS were reviewed. As summarized in Table II, for the individuals with MMIHS and

pathogenic variants in *ACTG2* (23 probands), the intestinal hypoperistalsis (21/21), and the bladder dysfunction are essential symptoms (23/23). The vesical hypocontractility often manifests as megacystis (91.3%— 21/23), and microcolon appears in 69.5% (16/23). Mydriasis and PDA were not identified in patients. The single reported individual with loss-of-function variant in *MYH11* presented with the typical signs of MMIHS. The main findings in individuals with MSMDS and proven pathogenic variants in *ACTA2* (probands) are mydriasis (23/23), PDA (22/23), and vascular abnormalities (22/23). Visceral dysfunction is mainly due to bladder involvement with (4/23) or without (10/23) megacystis, while intestinal hypoperistalsis is less frequent (5/23), and microlon is absent in patients with MSMDS. Although the prune-belly phenotype is not common, this feature was described in both phenotypes.

Discussion

In the present study, we report the clinical data and molecular investigation of seven individuals from six families presenting different phenotypes of visceral myopathy. Pathogenic variants were found in five families, each with only one affected individual.

In patients with MMIHS, we identified three individuals with heterozygous variants in *ACTG2*. All were sporadic and the respective variants have been previously described—c.532C>T [Thorson et al., 2014; Wangler et al., 2014; Halim et al., 2016], c.770G>A [Tuzovic et al., 2015], and c.533G>T [Thorson et al., 2014; Halim et al., 2016]. For the family with two siblings (typical phenotype of MMIHS and family history suggestive of AR pattern), the molecular investigation showed no pathogenic variants in *ACTG2*, *ACTA2*, or *MYH11*. Although we have identified 23 candidate genes by WES, a pathogenic variant could not yet be proven. Through the analysis of the exome, we did not find a pathogenic variation in the following genes: *CHRM3* (associated with urinary dysfunction and mydriasis in humans) [Weber et al., 2011], and other genes such as *CHRNA3*, *CHRNB2*, or *CHRNB4* (based on MMIHS-like phenotype—mydriais, megacystis, hypoperistalsis, gastric, and intestinal distention observed in mice) [Xu et al., 1999a,b].

Most of the published cases of MMIHS are sporadic and caused by de novo heterozygous variants in *ACTG2*. Recurrence in siblings related to *ACTG2* was recently reported under the hypothesis of gonadal mosaicism because the parents were asymptomatic and did not present the variant in the sequencing [Tuzovic et al., 2015]. In another patient, the variant in *ACTG2* had paternal origin, but the father had a milder phenotype [Wangler et al., 2014]. However, some individuals with MMIHS, but without pathogenic variant in *ACTG2* [Wangler et al., 2014; Halim et al., 2016], including two patients whose parents are consanguineous [Halim et al., 2016], suggest genetic heterogeneity and AR pattern in some. AR inheritance has been suggested because of the presentation of a number of families with recurrence in siblings and/or parental consanguinity, and asymptomatic parents [Mc Laughlin and Puri, 2013]. The molecular evidence supporting this inheritance pattern was recently demonstrated by Gauthier et al. [2014] in an individual with MMIHS and a homozygous variant (c.3598A>T—p.LysK1200Ter) in *MYH11*, whose parents are consanguineous. Although a functional study has not yet been performed, this variant was absent in the genome database, as well as in 323 control individuals. This variant is

predicted as likely to be deleterious by in silico analysis [Gauthier et al., 2014]. These authors defended the hypothesis that this variant could interfere with dimerization of the protein or inducing the degradation of the transcript through nonsense-mediated decay. The association of *MYH11* with MMIHS is supported by animal models in which the mice with homozygous deletion of *Myh11* presented with symptoms such as giant bladder, abnormal intestinal movement, and delay in the closure of ductus arteriosus [Morano et al., 2000].

The individual with CIPO has the novel heterozygous variant c.584C>T in *ACTG2*. Its pathogenicity was suggested by in silico analysis and reinforced by its absence in different databases, and in 100 alleles from control individuals.

Until now, 31 families with ACTG2-related disorders and 15 different pathogenic variants in *ACTG2* were described, all but one being missense variants—supplementary material (SM5) [Lehtonen et al., 2012; Holla et al., 2014; Thorson et al., 2014; Wangler et al., 2014; Klar et al., 2015; Tuzovic et al., 2015; Halim et al., 2016]. The only exception is an in tandem base substitution reported by Klar et al. [2015]. Many missense variants (10/14 = 71.4%) resulting in a substitution of an arginine for another amino acid in different codons [Lehtonen et al., 2012; Holla et al., 2014; Thorson et al., 2014; Wangler et al., 2014; Tuzovic et al., 2015; Halim et al., 2016].

Due to diversity of the manifestations of visceral myopathy related to *ACTG2*, Wangler et al. [2014] proposed a phenotypic spectrum of ACTG2-disorders ranging from MMIHS, the most severe presentation characterized by early onset of symptoms (prenatal or neonatal) with severe dysmotility of bowel and bladder, and parenteral nutrition dependence, to milder forms with variable degree of gastrointestinal, and urinary dysfunction, including CIPO, also referred as familial visceral myopathy [Lehtonen et al., 2012]. Indeed, CIPO was the first phenotype associated with *ACTG2* variants [Lehtonen et al., 2012]. It is not possible to define a genotype–phenotype correlation, since a same variant was observed in MMIHS and in individuals with a milder phenotype [Thorson et al., 2014; Wangler et al., 2014; Tuzovic et al., 2015; Halim et al., 2016]. However, cases resulting from de novo variants are frequently more severe than inherited cases [Wangler et al., 2014]. Although the penetrance seems to be complete, there is a considerable intra-familial variability and milder presentation may not be recognized [Lehtonen et al., 2012; Wangler et al., 2014]. Even in the severe phenotype, some variability related to the onset (prenatal or neonatal), voiding dysfunction and TPN dependence, have been observed [Wangler et al., 2014].

Beyond gastrointestinal and urological manifestations, others symptoms identified in individuals with pathogenic variants in *ACTG2*, including biliary complications (cholecystistis and cholelithiasis), and impaired uterine contraction, suggest that the myopathy may not be restricted to the gastrointestinal and urinary tracts, thus, expanding the phenotype [Lehtonen et al., 2012; Klar et al., 2015]. This is reinforced by the histologic findings of myopathy detected in the gallbladder of the patient with CIPO here reported.

In the patient with MSMDS, we identified a previously reported *ACTA2* variant, c.535C>T [Meuwissen et al., 2013]. Heterozygous variants in *ACTA2* were first described in individuals with thoracic aortic aneurysm with dissection (TAAD) [Guo et al., 2007],

coronary artery disease, stroke, and Moyamoya disease [Guo et al., 2009]. Then, a severe phenotype named multisystemic smooth muscle dysfunction syndrome (MSMDS) including PDA, congenital mydriasis, hypotonic bladder, ascending aorta aneurysms, and cerebrovascular disease was associated with a de novo heterozygous variant in the codon 179 [Milewicz et al., 2010]. The p.Arg179His variant is the most common substitution associated with this severe phenotype; however, other variants in the same codon (p.Arg179Cys and p.Arg179Lys) result in a similar phenotype [Moller et al., 2012; Munot et al., 2012; Meuwissen et al., 2013]. Patient 7, described in the present study with MSMDS, is the second individual identified with the c.535C>T (p.Arg179Cys) in *ACTA2.* The recurrent association between a severe clinical presentation and the arginine substitution in the 179 position suggests a genotype-phenotype correlation. The variant in this codon could cause a severe systemic disease because of the arginine in the 179 position, that is, near to a keyprotein–protein interaction site [Milewicz et al., 2010]. Interestingly, one individual reported by Roder et al. [2011] presenting the p.Arg179His variant has Moyamoya disease without other manifestations observed in MSMDS.

In order to better understand the overlap between MMIHS and MSMDS, two severe phenotypes with visceral myopathy, the review of published cases with proven pathogenic variants shown in Table II highlights more clearly the features characterizing each phenotype. While the visceral dysfunction in individuals with MMIHS manifests mainly as intestinal hypoperistalsis and megacystis, bladder involvement without megacystis is observed in patients with MSMDS. Some findings seem quite specific of each phenotype, as microcolon in MMIHS and mydriasis, PDA, and vascular abnormalities in MSMDS. Therefore, these findings may direct the molecular investigation of a patient with signs of visceral smooth muscle dysfunction. On the other hand, when the phenotype is more suggestive of MMIHS, there are no clear clinical signs for the differentiation between autosomal dominant *(ACTG2)* and recessive *(MYH11)* inheritance. Autosomal recessive inheritance seems to be less frequent than sporadic and autosomal dominant, and further studies are needed for a better etiologic definition of MMIHS. Although PDA had not been identified in individuals with homozygous *MYH11* variant [Gauthier et al., 2014], it remains a possibility due to involvement of ductus arteriosus in *Myh11* mice and in individuals with heterozygous variants in *MYH11* [Zhu et al., 2006]. Although the prune-belly appearance is not common in either MMIHS or MSMDS, this phenotype should be taken into account during the initial evaluation of a child with clinical signals of visceral motility dysfunction.

The main features present in MMIHS and MSMDS can be identified in other phenotypes such as the X-linked chronic intestinal pseudo-obstruction (CIPO-X), and urinary bladder disease/prune-belly caused by *FLNA* and *CHRM3* variants, respectively [Kapur et al., 2010; Weber et al., 2011]. In CIPO-X, besides CIPO, other manifestations including gastrointestinal abnormalities as intestinal malrotation, short small intestine, microcolon, and pyloric stenosis are described. Extra enteric findings like PDA, vascular abnormality in CNS, megacystis, periventricular nodular heterotopy, thrombocytopenia, and dysmorphic facies were observed in some patients [Clayton-Smith et al., 2009; Kapur et al., 2010]. The phenotype called urinary bladder disease/prune-belly, caused by homozygous *CHRM3* variants, is associated with bladder dysfunction, mydriasis, and dry mouths without intestinal abnormality [Weber et al., 2011].

The similarity of these diseases can be explained by the involvement of different parts of visceral contractile apparatus, including contractile filaments as α and γ-actin, myosin heavy chain 11, and filamin A (ACTA2, ACTG2, MYH11, and FLNA respectively), and muscarinic acetylcholine receptor (CHRM3). However, unexpected findings highlight interesting points regarding the expression of these proteins. For instance, the intestinal and bladder dysfunction in patients with *ACTA2* variants indicate that the α-actin isoform probably has a more important role in enteric smooth muscle cell than previously supposed, where the γ-actin is the major isoform [Milewicz et al., 2010]. The absence of vascular manifestations in the ACTG2-related disorders reinforces the concept that ACTG2 does not have an important function in vascular smooth muscle cell contraction.

With respect to mydriasis, it is usually described in MSMDS (*ACTA2*) as well as in urinary bladder disease/prune-belly (*CHRM3*). However, the pupils and their responses to light, and cholinergic agents seem to be different in these disorders. While the pupils in MSMDS are usually nonreactive to the light and the pilocarpine [Moller et al., 2012; Richer et al., 2012; Roulez et al., 2014], the pupils of the individuals with variants in *CHRM3* are described as having impaired constriction to light [Weber et al., 2011]. The presence of a weak light reflex and a very little change in pupil size when pilocarpine is instilled in *Chrm3* mutant mice support the hypothesis that other mechanisms could be related to the contraction of the pupillary sphincter muscle [Matsui et al., 2000]. Finally, despite CHRM3 being the main muscarinic receptor involved in visceral smooth muscle contraction, gastrointestinal symptoms were not evidenced in patients reported by Weber et al. [2011], suggesting a non-M3 acetylcholine receptor response or possible upregulated non cholinergic mechanisms of contraction maintaining gastrointestinal function, similar to those observed in animal models [Uchiyama and Chess-Williams, 2004].

In conclusion, a clinical and molecular study of seven individuals from six families with disorders of visceral smooth muscle contraction showed mutations in *ACTG2* (four) and in *ACTA2* (one) in five patients, and failed to find pathogenic variant, especially in *MYH11*, in the only consanguineous family, suggesting the existence of other gene(s) related to autosomal recessive inheritance. The results reinforce the clinical and genetic heterogeneity of the visceral myopathies. The review of MMIHS and MSMDS, similar phenotypes, suggest that some features can direct the molecular investigation of a patient with visceral myopathy. Finally, considering that the genes related to visceral myopathies in humans and/or in mouse models are genes encoding contractile proteins or acetylcholine receptors in enteric smooth muscle, further analysis should prioritize genes related to visceral contractile apparatus.

Addendum Added During Revision of the Manuscript

After the submission of this manuscript, two articles were published in on line version describing patients with ACTG2-related disorder:MMIHS [Lu et al.,2016] and MMIHS, and CIPO [Matera et al., 2016].

Web Sources

PolyPhen-2: http://genetics.bwh.harvard.edu/pph2/

Mutation Taster: http://www.mutationtaster.org/

MutPred: http://mutpred.mutdb.org/

Panther: http://www.pantherdb.org/

SNAP: https://rostlab.org/services/snap/

Phd-SNP and SNPs&GO: http://snps.biofold.org/snps-and-go/snps-and-go.html

dbSNP: http://www.ncbi.nlm.nih.gov/projects/SNP/

1000 Genomes project data: http://browser.1000genomes.org/index.html

Exome Variant Server: http://evs.gs.washington.edu/EVS/

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Grant sponsor: NHGRI; Grant number: 1U54HG006542; Grant sponsor: CNPq; Grant number: 590148/2011-7.

The authors thank the families for their participation in this study. The whole exome sequencing performed in this project was supported by NHGRI 1U54HG006542 to the Baylor-Hopkins Center for Mendelian Genomics.

References

Al-Mohaissen M, Allanson JE, O'Connor MD, Veinot JP, Brandys TM, Maharajh G, Dennie CJ, Beauchesne LM. Brachial artery occlusion in a young adult with an ACTA2 thoracic aortic aneurysm. Vasc Med. 2012; 17:326–329. [PubMed: 22946110]

Amans MR, Stout C, Fox C, Narvid J, Hetts SW, Cooke DL, Higashida RT, Dowd CF, McSwain H, Halbach VV. Cerebral arteriopathy associated with Arg179His ACTA2 mutation. J Neuro Interv Surg. 2014; 6:e46.

Brodsky MC, Turan KE, Khanna CL, Patton A, Kirmani S. Congenital mydriasis and prune belly syndrome in a child with an ACTA2 mutation. J AAPOS. 2014; 18:393–395. [PubMed: 24998021]

Clayton-Smith J, Walters S, Hobson E, Burkitt-Wright E, Smith R, Toutain A, Amiel J, Lyonnet S, Mansour S, Fitzpatrick D, Ciccone R, Ricca I, Zuffardi O, Donnai D. Xq28 duplication presenting with intestinal and bladder dysfunction and a distinctive facial appearance. Eur J Hum Genet. 2009; 17:434–443. [PubMed: 18854860]

Gauthier J, Ouled Amar Bencheikh B, Hamdan FF, Harrison SM, Baker LA, Couture F, Thiffault I, Ouazzani R, Samuels ME, Mitchell GA, Rouleau GA, Michaud JL, Soucy JF. A homozygous loss-of-function variant in MYH11 in a case with megacystis-microcolon-intestinal hypoperistalsis syndrome. Eur J Hum Genet. 2014; 23:1266–1268. [PubMed: 25407000]

Gosemann JH, Puri P. Megacystis microcolon intestinal hypoperistalsis syndrome: Systematic review of outcome. Pediatr Surg Int. 2011; 27:1041–1046. [PubMed: 21792650]

Guo DC, Pannu H, Papke CL, Yu RK, Avidan N, Bourgeois S, Estrera AL, Safi HJ, Sparks E, Amor D, Ades L, McConnell V, Willoughby CE, Abuelo D, Willing M, Lewis RA, Kim DH, Scherer S, Tung PP, Ahn C, Buja LM, Raman CS, Shete SS, Milewicz DM. Mutations in smooth muscle alpha-actin

(ACTA2) lead to thoracic aortic aneurysms and dissections. Nat Genet. 2007; 39:1488–1493. [PubMed: 17994018]

Guo DC, Papke CL, Tran-Fadulu V, Regalado ES, Avidan N, Johnson RJ, Kim DH, Pannu H, Willing MC, Sparks E, Pyeritz RE, Singh MN, Dalman RL, Grotta JC, Marian AJ, Boerwinkle EA, FrazierLQ, LeMaire SA, Coselli JS, Estrera AL, Safi HJ, Veeraraghavan S, Muzny DM, Wheeler DA, Willerson JT, Yu RK, Shete SS, Scherer SE, Raman CS, Buja LM, Milewicz DM. Mutations in smooth muscle alpha-actin (ACTA2) cause coronary artery disease, stroke, and moyamoya disease, along with thoracic aortic disease. Am J Hum Genet. 2009; 84:617–627. [PubMed: 19409525]

Halim D, Hofstra RM, Signorile L, Verdijk RM, van der Werf CS, Sribudiani Y, Brouwer RW, van IJcken WF, Dahl N, Verheij JB, Baumann C, Kerner J, van Bever Y, Galjart N, Wijnen RM, Tibboel D, Burns AJ, Muller F, Brooks AS, Alves MM. ACTG2 variants impair actin polymerization in sporadic Megacystis Microcolon Intestinal Hypoperistalsis Syndrome. Hum Mol Genet. 2016; 25:571–583. [PubMed: 26647307]

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]

Kapur RP, Robertson SP, Hannibal MC, Finn LS, Morgan T, van Kogelenberg M, Loren DJ. Diffuse abnormal layering of small intestinal smooth muscle is present in patients with FLNA mutations and x-linked intestinal pseudo-obstruction. Am J SurgPathol. 2010; 34:1528–1543.

Klar J, Raykova D, Gustafson E, Tóthová I, Ameur A, Wanders A, Dahl N. Phenotypic expansion of visceral myopathy associated with ACTG2 tandem base substitution. Eur J Hum Genet. 2015; 23:1679–1683. [PubMed: 25782675]

Lehtonen HJ, Sipponen T, Tojkander S, Karikoski R, Järvinen H, Laing NG, Lappalainen P, Aaltonen LA, Tuupanen S. Segregation of a missense variant in enteric smooth muscle actin *γ-2* with autosomal dominant familial visceral myopathy. Gastroenterology. 2012; 143:1482–1491. [PubMed: 22960657]

Lu W, Xiao Y, Wang J, Tao Y, Yan W, Lu L, Cao Y, Cai W. Mutation in actin γ-2 responsible for megacystis microcolon intestinal hypoperistalsis syndrome in four chinese patients. J Pediatr Gastroenterol Nutr. 2016; doi: 10.1097/MPG.0000000000001204

Matera I, Rusmini M, Guo Y, Lerone M, Li J, Zhang J, Di Duca M, Nozza P, Mosconi M, Prato AP, Martucciello G, Barabino A, Morandi F, De Giorgio R, Stanghellini V, Ravazzolo R, Devoto M, Hakonarson H, Ceccherini I. Variants of the ACTG2 gene correlate with degree of severity and presence of megacystis in chronic intestinal pseudoobstruction. Eur J Hum Genet. 2016; 24:1211–1215. [PubMed: 26813947]

Matsui M, Motomura D, Karasawa H, Fujikawa T, Jiang J, Komiya Y, Takahashi S, Taketo MM. Multiple functional defects in peripheral autonomic organs in mice lacking muscarinic acetylcholine receptor gene for the M3 subtype. Proc Natl Acad Sci USA. 2000; 97:9579–9584. [PubMed: 10944224]

Mc Laughlin D, Puri P. Familial megacystis microcolon intestinal hypoperistalsis syndrome: A systematic review. Pediatr Surg Int. 2013; 29:947–951. [PubMed: 23955298]

Meuwissen ME, Lequin MH, Bindels-de Heus K, Bruggenwirth HT, Knapen MF, Dalinghaus M, de Coo R, van Bever Y, Winkelman BH, Mancini GM. ACTA2 mutation with childhood cardiovascular, autonomic and brain anomalies and severe outcome. Am J Med Genet Part A. 2013; 161A:1376–1380. [PubMed: 23613326]

Milewicz DM, Østergaard JR, Ala-Kokko LM, Khan N, Grange DK, Mendoza-Londono R, Bradley TJ, Olney AH, Adès L, Maher JF, Guo D, Buja LM, Kim D, Hyland JC, Regalado ES. De novo ACTA2 mutation causes a novel syndrome of multisystemic smooth muscle dysfunction. Am J Med Genet Part A. 2010; 152A:2437–2443. [PubMed: 20734336]

Moller HU, Fledelius HC, Milewicz DM, Regalado ES, Ostergaard JR. Eye features in three Danish patients with multisystemic smooth muscle dysfunction syndrome. Br J Ophthalmol. 2012; 96:1227–1231. [PubMed: 22790431]

Morano I, Chai GX, Baltas LG, Lamounier-Zepter V, Lutsch G, Kott M, Haase H, Bader M. Smooth-muscle contraction without smooth-muscle myosin. Nat Cell Biol. 2000; 2:371–375. [PubMed: 10854329]

Moosa AN, Traboulsi EI, Reid J, Prieto L, Moran R, Friedman NR. Neonatal stroke and progressive leukoencephalopathy in a child with an ACTA2 mutation. J Child Neurol. 2013; 28:531–534. [PubMed: 22752479]

Munot P, Saunders DE, Milewicz DM, Regalado ES, Ostergaard JR, Braun KP, Kerr T, Lichtenbelt KD, Philip S, Rittey C, Jacques TS, Cox TC, Ganesan V. A novel distinctive cerebrovascular phenotype is associated with heterozygous Arg179 ACTA2 mutations. Brain. 2012; 135:2506–2514. [PubMed: 22831780]

Richer J, Milewicz DM, Gow R, de Nanassy J, Maharajh G, Miller E, Oppenheimer L, Weiler G, O'Connor M. R179H mutation in ACTA2 expanding the phenotype to include prune-belly sequence and skin manifestations. Am J Med Genet Part A. 2012; 158A:664–668. [PubMed: 22302747]

Roulez FM, Faes F, Delbeke P, Van Bogaert P, Rodesch G, De Zaeytijd J, Depasse F, Coucke PJ, Meire FM. Congenital fixed dilated pupils due to ACTA2 multisystemic smooth muscle dysfunction syndrome. J Neuroophthalmol. 2014; 34:137–143. [PubMed: 24621862]

Roder C, Peters V, Kasuya H, Nishizawa T, Wakita S, Berg D, Schulte C, Khan N, Tatagiba M, Krischek B. Analysis of ACTA2 in european moyamoya disease patients. Europ J Paedit Neurol. 2011; 15:117–122.

Sobreira N, Schiettecatte F, Boehm C, Valle D, Hamosh A. New tools for Mendelian disease gene identification: PhenoDB variant analysis module and GeneMatcher, a web-based tool for linking investigators with an interest in the same gene. Hum Mutat. 2015; 36:425–431. [PubMed: 25684268]

Thorson W, Diaz-Horta O, Foster J 2nd, Spiliopoulos M, Quintero R, Farooq A, Blanton S, Tekin M. De novo ACTG2 mutations cause congenital distended bladder, microcolon, and intestinal hypoperistalsis. Hum Genet. 2014; 133:737–742. [PubMed: 24337657]

Tuzovic L, Tang S, Miller RS, Rohena L, Shahmirzadi L, Gonzalez K, Li X, LeDuc CA, Guo J, Wilson A, Mills A, Glassberg K, Rotterdam H, Sepulveda AR, Zeng W, Chung WK, Anyane-Yeboa K. New insights into the genetics of FMegacystis: ACTG2 mutations, encoding γ-2 smooth muscle actin in megacystis microcolon intestinal hypoperistalsis syndrome (Berdon syndrome). Fetal Diagn Ther. 2015; 38:296–306. [PubMed: 25998219]

Uchiyama T, Chess-Williams R. Muscarinic receptor subtypes of the bladder and gastrointestinal tract. J Smooth Muscle Res. 2004; 40:237–247. [PubMed: 15725706]

Venselaar H, TeBeek TA, Kuipers RK, Hekkelman ML, Vriend G. Protein structure analysis of mutations causing inheritable diseases. An e-Science approach with life scientist friendly interfaces. BMC Bioinformatics. 2010; 11:548–557. [PubMed: 21059217]

Wangler MF, Gonzaga-Jauregui C, Gambin T, Penney S, Moss T, Chopra A, Probst FJ, Xia F, Yang Y, Werlin S, Eglite I, Kornejeva L, Bacino CA, Baldridge D, Neul J, Lehman EL, Larson A, Beuten J, Muzny DM, Jhangiani S, Baylor-Hopkins Center for Mendelian Genomics. Gibbs RA, Lupski JR, Beaudet A. Heterozygous de novo and inherited mutations in the smooth muscle actin (ACTG2) gene underlie megacystis-microcolon-in-testinal hypoperistalsis syndrome. PLoS Genet. 2014; 10:e1004258. [PubMed: 24676022]

Weber S, Thiele H, Mir S, Toliat MR, Sozeri B, Reutter H, Draaken M, Ludwig M, Altmüller J, Frommolt P, Stuart HM, Ranjzad P, Hanley NA, Jennings R, Newman WG, Wilcox DT, Thiel U, Schlingmann KP, Beetz R, Hoyer PF, Konrad M, Schaefer F, Nürnberg P, Woolf AS. Muscarinic acetylcoline receptor M3 mutation causes urinary bladder disease and a prune-Belly-like syndrome. Am J Hum Genet. 2011; 89:668–674. [PubMed: 22077972]

Xu W, Gelber S, Orr-Urtreger A, Armstrong D, Lewis RA, Ou CN, Patrick J, Role L, De Biasi M, Beaudet AL. Megacystis, mydriasis, and ion channel defect in mice lacking the alpha3 neuronal nicotinic acetylcho-line receptor. Proc Natl Acad Sci USA. 1999a; 96:5746–5751. [PubMed: 10318955]

Xu W, Orr-Urtreger A, Nigro F, Gelber S, Sutcliffe CB, Armstrong D, Patrick JW, Role LW, Beaudet AL, De Biasi M. Multiorgan autonomic dysfunction in mice lacking the beta2 and the beta4 subunits of neuronal nicotinic acetylcholine receptor. J Neurosci. 1999b; 19:9298–9305. [PubMed: 10531434]

Yetman AT, Starr LJ, Bleyl SB, Meyers L, Delaney JW. Progressive aortic dilation associated with ACTA2 mutations presenting in infancy. Pediatrics. 2015; 136:e262–e266. [PubMed: 26034244]

Zhu L, Vranckx R, Khau Van Kien P, Lalande A, Boisset N, Mathieu F, Wegman M, Glancy L, Gasc JM, Brunotte F, Bruneval P, Wolf JE, Michel JB, Jeunemaitre X. Mutations in myosin heavy chain 11 cause a syndrome associating thoracic aortic aneurysm/aortic dissection and patent ductus arteriosus. Nat Genet. 2006; 38:343–349. [PubMed: 16444274]

Table I

Clinical and Molecular Findings of the Seven Evaluated Patients

	Family 1		Family 2	Family 3	Family 4	Family 5	Family 6
	Patient 1	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**	**Patient 6**	**Patient *7***
Phenotype	MMIHS	MMIHS	MMIHS	MMIHS	MMIHS	CIPO	MSMDS
Parental consanguinity	+	+	–	–	–	–	–
Familial recurrence	+ (Patient 2)	+ (Patient 1)	–	–	–	–	–
Pathogenic variant							
*ACTG2**	NE	Not identified	C.532c>T (p.Argl78Cys) Het—de novo	c770G>A (p.Arg257His) Het—de novo	.533G>T (p.Arg178Leu) Het—de novo	c.584C>T (p.Thr195lle) Het— unknown origin	Not identified
*ACTA2**	NE	Not identified	NE	NE	NE	NE	c. 535c>T (p.Arg179Cys) Het—unknown origin
*MYH11***	NE	Not identified	NE	NE	NE	NE	NE
Gender	M	F	M	F	M	F	F
Newborn data vitality	Live	Live	Live	Live	Live	Live	Live
Weight [g] (centile)	1,935 (<p10)	4,150 (>90)	2,600 (10–25)	3,360 (50–75)	3,025 (75–90)	3,320 (50–75)	3,690 (75–90)
Length [cm] (centile)	38 (<p10)	50 (50–75)	42 (10)	50 (25–50)	49 (75–90)	NS	48 (10–25)
OFC [cm] (centile]	31 (p10)	34.5 (50–75)	32 (25)	36 (90)	33 (50–75)	NS	35 (75)
Gestational age [weeks]	36	38	36	39	35	*37*	38
Apgar score	3/8	9/10	*7/10*	9/10	8/9	NE	8/9/9
Age	Death (1 mo)	Death (1 y 8 mo)	Death (7 mo)	Death (11 mo)	5 mo	5y	2 y 10 mo
Paternal age (years)[a]	21	23	30	38	32	34	38
Maternal age (years)[a]	24	2B	29	34	25	*27*	29
Prenatal findings	Hydronephrosis, megacystis, oligohydramnios	Hydronephrosis, megacystis, polyhydramnios	Hyperechogenic kidneys, hydroureter, megacystis, club feet	Megacystis, polyhydramnios	Megacystis	Megacystis	Hydronephrosis, hydroureter, megacystis
Fetal bladder diversion	Vesicoamniotic shunt	–	–	–	–	–	–

	Family 1		Family 2	Family 3	Family 4	Family 5	Family 6
	Patient 1	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**	**Patient 6**	**Patient *7***
Megacystis	+	+	+	+	+	Episodic	+
Prune-belly phenotype	+	+	+	–	–	–	–
Microcolon	+	+	+	–	+	–	–
Intestinal hypoperistalsis	+	+	+	+	+	+	+
Mydriasis	NE	+	–	–	–	–	+
Parenteral lnutrition dependence	+ (Total)	+ (Total)	+ (Total)	+ (Total after 4 mo)	+ (Total)	+ (Intermittent)	+ (Intermittent)
Bladder catheterization and/or vesicostomy	+	+	+	+	+	–	+
Other findings	Hydronephrosis, sparse renal cyst, renal failure, dilated stomach, intestinal malrotation, hypoplastic terminal ileum	Hydronephrosis, intestinal malrotation, hypoplastic small intestine	Hydronephrosis, renal cysts, hydroureter, stomach dilatation		Cerebral ventriculomegaly, temporal epileptiform discharges	Stomach and large intestine dilatation	Hydronephrosis, hydroureter, intestinal malrotation, ASD, ductus arteriosus aneurysm, pulmonary valve dilatation, cerebrovascular abnormalities
Histologic findings	Renal dysplasia, chronic pyelonephritis, acute tubular necrosis, intrahepatic cholestasis	Vacuolization of muscularis propria layer (bladder and small intestine)	NE	Normal (stomach, small and large intestine)	Enterocolitis	Vacuolization and fibrosis of muscularis propria layer (stomach, gallbladder, small and large intestine)	Normal (large intestine)
Surgery	Exploratory laparotomy, gastrostomy, ileostomy, vesicostomy, appendicectomy	Gastrostomy, ileostomy, vesicostomy, multivisceral transplantation	Ileostomy, vesicostomy	Gastrostomy, ileostomy	Ileostomy	Ileostomy, partial gastrectomy with Roux loop anatomosis, cholecystectomy, exploratory laparotomy, right hemicolectomy	Ligation of ductus arteriosus, gastrostomy, sigmoid resection
Follow up	Deceased	Deceased	Deceased	Deceased	Supportive treatment	Episodic intestinal pseudo-obstruction, supportive treatment	Supportive treatment

[a] parental age in years at the birth of the patient; ASD, atrial septal defect; CIPO, chronic intestinal pseudo-obstruction; F, female; Het, heterozygous; M, male; MMIHS, megacystis-microcolon-intestinal hypoperistalsis; mo, months old; MSMDS, i smooth muscle dysfunction syndrome; NE, not evaluated; OFC, occipital frontal circumference; y, years old; +, feature present; —, feature absent.

[*] Evaluated by Sanger sequencing in patients *2-7* (both genes were also tested by whole-exome sequencing in patient *2*).

Author Manuscript

Author Manuscript

Author Manuscript

** Evaluated by whole-exome sequencing in patient 2.

Table II

MMIHS and MSMDS: Summary of Clinical Data and Genotype of Individuals With Molecularly Confirmed Diagnosis

Phenotype	Megacystis-microcolon-intestinal hypoperistalsis syndrome (MMIHS)		Multisystemic smooth muscle dysfunction syndrome (MSMDS)
Gene	*ACTG2*[a]	*MYH11*[b]	*ACTA2*[c]
Number of probands	23	1	23
Parental consanguinity	−	+ (Algerian origin)	−
Inheritance	AD	AR	AD
Pathogenic variant	Heterozygous—all missense	Homozygous—stop codon	Heterozygous—all missense (codon 179)
De novo variant	16/18[d]	No	23/23
Familial recurrence of the phenotype	1/23[e]	−	−
Megacystis	21/23	+	4/23
Bladder dysfunction without megacystis	2/23	−	10/23
Microcolon	16/23	+	−
Intestinal hypoperistalsis	21/21[f]	+	5/23
Prune-belly	2/20[g]	+	2/23
Mydriasis	−	−	23/23
Patent ductus arteriosus	−	−	23/23
Vascular involvement			23/23
Cerebrovascular	−	−	21/23
Aortic aneurism	−	−	14/23

AD, autosomal dominant, AR, autosomal recessive, MMIHS, megacystis-microcolon-intestinal hypoperistalsis syndrome; MSMDS, multisystemicsmooth muscle dysfunction syndrome; +, feature present; —, feature absent. A summary of phenotype, genotype, and family history data of all individuals with ACTG2-related disorder, including MMIHS and milder phenotypes, is described in the supplementary material (SM5).

[a] [Thorson et al., 2014]; [Wangler et al., 2014]; [Tuzovic et al., 2015]; [Halim et al., 2016].

[b] [Gauthier et al., 2014].

[c] [Milewicz et al., 2010]; [Al-Mohaissen et al., 2012]; [Moller et al., 2012]; [Munot et al., 2012]; [Richer et al., 2012]; [Meuwissen et al., 2013]; [Moosa et al., 2013]; [Amans et al., 2014]; [Brodsky et al., 2014]; [Roulez et al., 2014]; [Yetman et al., 2015].

[d] Unknown finding in five of the 23 individuals (18 evaluated patients), 16/18, one individual inherited the variant from the father, who presented with milder disease—family 34 [Wangler et al., 2014] and parental inheritance in a family due to probable gonadal mosacism—family 1 [Tuzovic et al., 2015].

[e] The recurrence of MMIHS was reported in a family with two affected siblings and normal parents—family 1 [Tuzovic et al., 2015].

[f] Unknown finding in two of the 23 individuals (21 evaluated patients).

[g] Unknown finding in three of the 23 individuals (20 evaluated patients).