Supplementary material (SM)

SM1 – Information about Sanger sequencing of *ACTG2* and *ACTA2*.

The genomic DNA was amplified by the polymerase chain reaction (PCR), the PCR products were sequencing using BigDyeTerminator Cycle Sequencing Kit and ABI 3500 xL sequencer (Applied Biosystems, Life Technologies Corporation), and the sequence analysis was accomplished by CodonCode Aligner V5.0.2. The primers used are listed in the table S1.They were designed using Gene Runner program V.3.05.

TABLE S1 – Primers used in PCR and Sanger sequencing

***ACTG2* Exon**	**Primer foward**	**Primer reverse**	**Ta (°C)**	**PCR Size (bp)**
2	catttcagggcagagggag	gtgtgcctgatgtatgttgg	54.1	626
3	cctccaccttctcttcctg	ggttggagaaatggtcgtg	54.1	478
4	ctgtaggagagtgaagaggagg	cattattccacacccactcc	54.1	541
5	agtgggtgtggaataatgaac	gtgtatgtgtagaagctcctgg	54.1	469
6	gattgatgattcttgtgatggg	gatcttcactaccattcagcctg	54.1	571
7	gaataaagtagatgtgagaaggagg	cttgctacattgctgcctac	54.1	585
8	gcaacaatcgaagaagggtc	gtcttcagcccatcaatgc	54.1	455
9	cacaatgcccagccttac	CCTTGACTTCTCTGAGCCTC	54.1	684
***ACTA2* Exon**	**Primer foward**	**Primer reverse**	**Ta (°C)**	**PCR Size (bp)**
2	gatagcactaggacacagagg	tggcatttactcctggacc	52,8	422
3	gctatagtgatggacaagacagac	cagttgagcaatgtgagcc	52,8	533
4	ggtacttacacactttgagcttc	gctgttcctgtcctttggg	52,8	445
5	ctgaacctccttactttggc	ggagaggtgaggtgggac	58,8	480
6	gtcccacctcacctctcc	gatgtgctttgctctgtgc	52,8	425
7	ggcacatgtcttgagggag	cacccttgctacccctttcc	54,1	587
8	gtcttcccagcctcagtc	gaaagtagctgacagacaagg	54,1	531
9	ccctctggcggtcctg	ggaccactagagggagcc	52,8	565

SM2 – Information about whole-exome sequencing

The Agilent SureSelect Human All Exon V4 - 51Mb kit (Agilent Technologies, Santa Clara, CA) was used to capture the target regions. Genomic DNA (1 ug) was sheared using the Covaris E210 instrument (Covaris) followed by end repair, A-tailing and adapter ligation. A hybrid protocol for library preparation and whole exome enrichment was based on methods and parameters from Fisher et al. [2011] applied to the reagents, volumes and parameters from de Agilent SureSelect XT kit and automated protocol. The WES (paired end 100 bp reads) was performed on the patient 2 and her parents using the Illumina HiSeq2500 platform (Illumina, Inc. San Diego, CA). Each read was aligned to the 1000 genomes phase 2 (GRCh37) human genome reference using the Burrows–Wheeler Alignment (BWA) version 0.7.8 [Li H., 2013]. PCR duplicates were removed using the Picard software version 1.109. Local realignment around indels and base call quality score recalibration were performed using the Genome Analysis Toolkit (GATK) [McKenna et al., 2010] version 3.1-1-g07a4bf8. Variant filtering was done using the Variant Quality Score Recalibration (VQSR) method [DePristo et al., 2011]. SNVs and indels were filtered to obtain all variants up to the 99th percentile of truth sites (0,1% false negative rate). The phenoDB analysis tool was applied to filter and prioritize rare functional variants (missense, nonsense, splice site variants, and indels) [Sobreira et al., 2015].

SM3 – Figure 1. Molecular analysis (DNA chromatograms) with identified pathogenic variants in the *ACTG2* (A, B, C and D) and *ACTA2* (E) - A: patient 3 (c.532C>T - p.Arg178Cys), B: patient 4 (c.770G>A – p.Arg257His), C: patient 5 (c.533G>T – p.Arg178Leu), D: patient 6 (c.584C>T - p.Thr195Ile) and E: patient 7 (c.535C>T – p.Arg179Cys). The variants were not identified in the evaluated parents.

SM4 – Pathogenicity of c.584C>T variant found in *ACTG2* (patient 6) predicted by different softwares

Poly-Phen: possibly damaging (0.91).

Mutation Taster: disease (89).

Mutpred: probability of deleterious mutation: 0.78 (confident hypotheses).

Panther: disease (probability: 0,64).

Phd-SNP: disease (probability: 0,59).

Neutral variant: SNAP and SNPs&GO.

SM5 - Information about the individuals with molecularly confirmed ACTG2-related disorders

TABLE SII. Summary of phenotype, genotype and family history data of individuals with molecularly confirmed ACTG2-related disorders

Proband (P)	Number of individuals molecularly confirmed in the family	Phenotype	Variant	Inheritance	Onset (identification of symptoms)	Megacystis (time at diagnosis)	Voiding dysfunction (therapy)	Microcolon	Gastrointestinal dysfunction	Parenteral nutrition dependency	Prune-belly
Lehtonen et al., 2012											
P1	8	CIPO	c.442C>A: p.Arg148Ser	familial	11y-20y	-	1/8 (x)	-	8/8	1/8 (intermittent)	-
Holla et al., 2014											
P1	1*	CIPO	c.442C>A: p.Arg148Ser	familial*	adult	-	-	-	+	+ (intermittent)	-
Thorson et al., 2014											
P1	1	MMIHS	c.533G>T: p.Arg178Leu	*de novo*	postnatal	+ (postnatal)	+ (bladder cathet)	-	+	+	-
P2	1	MMIHS	c.532C>T: p.Arg178Cys	*de novo*	prenatal	+ (prenatal)	+ (vesicostomy)	+	+	x	-
Wangler et al., 2014											
P1 (Fam4)	1	MMIHS	c.769C>T: p.Arg257Cys	*de novo*	postnatal	+ (postnatal)	+ (bladder cathet)	-	+	+	-
P2 (Fam11)	2	not specified	c.533G>A: p.Arg178His	familial	x	x	x	x	x	x	x
P3 (Fam12)	1	HVM	c.119G>A: p.Arg40His	*de novo*	prenatal	+ (prenatal)	+ (bladder cathet)	-	+	-	-
P4 (Fam13)	2	CIPO	c.769C>T: p.Arg257Cys	familial	prenatal [1/2]	+ (prenatal)	x	x	2/2	x	x
P5 (Fam14)	1	MMIHS	c.134T>C: p.Met45Thr	*de novo*	postnatal	-	+ (bladder cathet)	+	+	+ (intermittent)	-
P6 (Fam16)	1	MMIHS	c.400T>A: p.Tyr134Asn	*de novo*	prenatal	+ (prenatal)	+ (vesicostomy)	-	+	+	+
P7 (Fam17)	1	not specified	c.187C>G: p.Arg63Gly	x	x	x	x	x	x	x	x
P8 (Fam19)	2	CIPO	c.330C>A: p.Phe110Leu	familial	x	-	-	-	1/2	2/2	-
P9 (Fam25)	1	MMIHS	c.769C>T: p.Arg257Cys	*de novo*	prenatal	+ (prenatal)	+ (bladder cathet)	+	+	+	-
P10 (Fam26)	1	MMIHS	c.118C>T: p.Arg40Cys	*de novo*	prenatal	+ (prenatal)	+ (bladder cathet)	+	+	+ (intermittent)	-
P11 (Fam28)	1	MMIHS	c.593G>A: p.Gly198Asp	*de novo*	postnatal	-	+ (bladder cathet)	-	+	+	-
P12 (Fam29)	1	MMIHS	c.532C>T: p.Arg178Cys	*de novo*	prenatal	+ (prenatal)	+ (bladder cathet)	+	+	+	-

Proband (P)	Number of individuals molecularly confirmed in the family	Phenotype	Variant	Inheritance	Onset (identification of symptoms)	Megacystis (time at diagnosis)	Voiding dysfunction (therapy)	Microcolon	Gastrointestinal dysfunction	Parenteral nutrition dependency	Prune-belly
P13 (Fam30)	1	MMIHS	c.769C>T: p.Arg257Cys	*de novo*	prenatal	+ (prenatal)	+ (bladder cathet)	-	+	+	-
P14 (Fam34)	6	MMIHS [1] CIPO [5]	c.119G>A: p.Arg40His	familial	prenatal [1/6]	1/6 (prenatal)	1/6 (bladder cathet)	-	6/6	-	-
P15 (Fam35)	1	MMIHS	c.533G>A: p.Arg178His	*de novo*	prenatal	+ (prenatal)	+ (bladder cathet)	+	+	+	-
Tuzovic et al., 2015											
P1	2**	MMIHS	c.770G>A: p.Arg257His	gonadal mosaicism?	2/2 (prenatal)	2/2 (prenatal)	2/2 (bladder cathet)	1/2	2/2	1/2	-
P2	1	MMIHS	c.533G>A: p.Arg178His	*de novo*	+ (prenatal)	+ (prenatal)	+ (bladder cathet)	+	+	+	-
P3	1	MMIHS	c.769C>T: p.Arg257Cys	*de novo*	+ (prenatal)	+ (prenatal)	+ (bladder cathet)	-	+	+	-
Klar et al., 2015											
P1	7***	CIPO	c.806_807delinsAA: p.Gly269Glu	familial	1m-16y	7/7 (x)	6/7 (x)	-	7/7	-	-
Halim et al., 2015											
P1 (S1)	1	MMIHS	c.187C>G: p.Arg63Gln	x	prenatal	+ (prenatal)	x	+	+	x	-
P2 (S2)	1	MMIHS	c.533G>A: p.Arg178His	*de novo*	x	+ (x)	x	+	+	x	x
P3 (S3)	1	MMIHS	c.532C>T: p.Arg178Cys	*de novo*	prenatal	+ (x)	x	+	+	x	+
P4 (S4)	1	MMIHS	c.533G>A: p.Arg178His	x	prenatal	+ (prenatal)	x	+	+	x	-
P5 (S5)	1	MMIHS	c.533G>T: p.Arg178Leu	*de novo*	prenatal	+ (prenatal)	+ (bladder cathet)	+	+	x	-
P6 (S6)	1	MMIHS	c.118C>T: p.Arg40Cys	x	prenatal	+ (prenatal)	x	+	+	+ (partial)	-
P7 (S7)	1	MMIHS	c.532C>T: p.Arg178Cys	x	prenatal	+ (x)	x	+	x	x	x
P8 (S8)	1	MMIHS	c.532C>T: p.Arg178Cys	x	prenatal	+ (x)	x	+	x	x	x

bladder cathet: bladder catheterization; CIPO: chronic intestinal pseudo-obstruction; Fam: family – description based on original article; HVM: hollow visceral myopathy; m: months old; MMIHS: megacystis-microcolon-intestinal hypoperistalsis syndrome; S: sporadic case – description based on original article; y: years old; +: feature present; -: feature absent; x: unknown or not reported

*: five affected individuals but only the proband was molecularly confirmed with ACTG2-related disorder; ** The hypothesis of gonadal mosaicism was considered in a family with two affected siblings and normal parents; *** 12 affected individuals but only seven individuals were molecularly confirmed with ACTG2-related disorder

REFERENCES

DePristo M, Banks E, Poplin R, Garimella K, Maguire J, Hartl C, Philippakis A, del Angel G, Rivas MA, Hanna M, McKenna A, Fennell T, Kernytsky A, Sivachenko A, Cibulskis K, Gabriel S, Altshuler D and Daly, M. 2011. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nature Genetics 43:491-498.

Fisher S, Barry A, Abreu J, Minie B, Nolan J, Delorey TM, Young G, Fennell TJ, Allen A, Ambrogio L, Berlin AM, Blumenstiel B, Cibulskis K, Friedrich D,Johnson R, Juhn F, Reilly B, Shammas R, Stalker J, Sykes SM, Thompson J, Walsh J, Zimmer A, Zwirko Z, Gabriel S, Nicol R, Nusbaum C. 2011. A scalable, fully automated process for construction of sequence-ready human exome targeted capture libraries. Genome Biology 12:R1.

Li H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv:1303.3997v1 [q-bio.GN].

McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA. 2010. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20:1297-1303.

Sobreira N, Schiettecatte F, Boehm C, Valle D, Hamosh A. 2015. Hum Mutat 36:425-431.