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Variants in *ACTG2* underlie a substantial number of Australasian patients with primary chronic intestinal pseudo-obstruction

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Short running title:
ACTG2 in chronic intestinal pseudo-obstruction

Abstract

Background: Primary chronic intestinal pseudo-obstruction is a rare, potentially life-threatening disorder characterised by severely impaired gastrointestinal motility. The objective of this study was to examine the contribution of *ACTG2*, *LMOD1*, *MYH11* and *MYLK* mutations in an Australasian cohort of patients with a diagnosis of primary chronic intestinal pseudo-obstruction associated with visceral myopathy.

Methods: Paediatric and adult patients with primary chronic intestinal pseudo-obstruction and suspected visceral myopathy were recruited from across Australia and New Zealand. Sanger sequencing of the genes encoding enteric gamma-actin (*ACTG2*) and smooth muscle leiomodin (LMOD1) was performed on DNA from patients, and their relatives, where available. *MYH11* and *MYLK* were screened by next generation sequencing.

Key results: We identified heterozygous missense variants in *ACTG2* in seven of 17 families (~41%) diagnosed with chronic intestinal pseudo-obstruction and its associated conditions. We also identified one previously unpublished missense mutation (c.443C>T, p.Arg148Leu) in one family. One case presented with megacystis-microcolon-intestinal hypoperistalsis syndrome *in utero* with subsequent termination of

pregnancy at 28 weeks gestation. All of the substitutions identified occurred at arginine residues. No likely pathogenic variants in *LMOD1*, *MYH11* or *MYLK*, were identified within our cohort.

Conclusions and inferences: *ACTG2* mutations represent a significant underlying cause of primary chronic intestinal pseudo-obstruction with visceral myopathy and associated phenotypes in Australasian patients. Thus *ACTG2* sequencing should be considered in cases presenting with hypoperistalsis phenotypes with suspected visceral myopathy. It is likely that variants in other genes encoding enteric smooth muscle contractile proteins will contribute further to the genetic heterogeneity of hypoperistalsis phenotypes.

Keywords: *ACTG2*, chronic intestinal pseudo-obstruction, visceral myopathy

Key points:

- Mutations in *ACTG2*, *LMOD1*, *MYH11* and *MYLK* cause primary CIPO. Here we examined the contribution of these genes within an Australasian cohort of patients presenting with primary CIPO with suspicion of a visceral myopathy.
- We identified mutations in *ACTG2* in seven of 17 probands, this included identification of one unpublished substitution (p.Arg148Leu). We did not identify mutations in *LMOD1*, *MYH11* or *MYLK*.
- *ACTG2* mutations should be considered in suspected visceral myopathy patients, regardless of whether there is a family history, due to the high rate of *de novo* mutation.

1. INTRODUCTION

Chronic intestinal pseudo-obstruction (CIPO) is a debilitating disorder, characterised by severe impairment of gastrointestinal motility and clinical features suggestive of obstruction in the absence of an organic blockage (1, 2). Primary or secondary forms of CIPO are described, based on whether CIPO occurs in isolation or as a consequence from another disease state. A variety of underlying neuromuscular causes have been implicated in primary CIPO, including intrinsic or extrinsic neuropathies, myopathies, and abnormalities of the interstitial cells of Cajal; which may be found in isolation or in

combination. Most cases of primary CIPO are sporadic, however familial forms have also been reported (3, 4).

The term 'visceral myopathy' (degenerative leiomyopathy) has been used to describe the pathological changes underlying a subset of CIPO patients, which may be seen in familial forms. This is a rare group of disorders characterised by hereditary degeneration of potentially both gastrointestinal and urinary tract smooth muscle. It classically presents after the first decade of life with megaduodenum, megacystis and symptoms such as abdominal distension and pain, vomiting, constipation, diarrhoea, dysphagia, and recurrent urinary tract infections. A large Finnish kindred presenting with familial visceral myopathy was described in 2009; intestinal biopsies showed alpha-actin positive inclusions(3). Subsequently, a variant was identified in the gene encoding enteric gamma-actin (*ACTG2*, exon 5, c.442C>A, p.Arg148Ser)(5). Since this study, a number of cohort studies and case reports have described *de novo* or dominantly inherited *ACTG2* variants in patients with hypoperistalsis disorders(6-11).

Recently, an individual with severe visceral myopathy and a homozygous truncating variant (c.502C>T, p.Arg168*) in *ACTG2* was reported(12). This patient had severe recurrent intestinal obstruction necessitating intestinal transplant. The heterozygous carrier parents and siblings had a history of chronic constipation(12). Variants of *ACTG2* have also been identified in patients with megacystis-microcolon-intestinal hypoperistalsis syndrome (MMIHS; OMIM 155310), which is characterised by prenatal onset bladder distension and intestinal pseudo-obstruction (6-8)

Beyond *ACTG2*, a number of other specific genetic variants are now recognised as potentially giving rise to CIPO-associated phenotypes by severely impacting smooth muscle function, including variants in *MYH11, LMOD1*, and *MYLK*. The role of *MYH11* was recognised by Gauthier *et al.* who described an isolated case of MMIHS and prune belly syndrome, and identified a homozygous missense variant (13). This gene encodes smooth muscle myosin heavy chain. In two recent studies, Halim and colleagues identified homozygous loss-of-function variants in *LMOD1*(14) and *MYLK*(15), in cases presenting with MMIHS. *LMOD1* (OMIM 602715) encodes smooth muscle leiomodin, Halim *et al.* showed that *Lmod1* null mice presented with a similar phenotype. Bladder

distension was evident in late-stage embryos and stomach enlargement was present post feeding. Histology revealed thinning and compaction of the visceral smooth muscle of the stomach and bladder(14). Biallelic variants of *LMOD3*, which encodes a skeletal muscle-specific leiomodin, cause a severe nemaline myopathy(16). *MYLK* (OMIM 600922) encodes a smooth muscle myosin light chain kinase. Phosphorylation of the myosin light chain is the first step involved in smooth muscle contraction and is mediated by MYLK. Interestingly, heterozygous variants of *LMOD1*(17), *MYH11* (OMIM 132900) and *MYLK* (OMIM 613780) cause aortic aneurysms and dissections.

The increasing recognition of specific genetic defects in patients with primary CIPO and severe smooth muscle dysfunction provides the opportunity to move beyond clinical or pathological descriptors, in order to achieve specific root-cause disease classifications and precise diagnostics. In this study, we therefore aimed to investigate the contribution of *ACTG2*, *LMOD1*, *MYLK* and *MYH11* variants to disease in a cohort of patients presenting with primary CIPO and suspected smooth muscle dysfunction from Australia and New Zealand.

2. PATIENTS AND METHODS

2.1 Participants

The Department of Diagnostic Genomics at PathWest Laboratory Medicine (Western Australia) provides a high-volume diagnostics and research service for muscle disorder genetics in Australasia. Participants in this study were patients with a clinical diagnosis of primary CIPO and who were suspected to have a visceral myopathy (degenerative leiomyopathy) or associated phenotype. Referring clinicians provided comprehensive summaries of clinical data on a standardised proforma for clinicopathological correlation, and provided DNA samples to the Department of Diagnostic Genomics. Diagnoses were clinically-determined by gastroenterological, surgical or genetics specialists at hospital sites across Australia and New Zealand. All consecutive patients over the recent five-year period (2013-2017) were included.

Primary CIPO with a suspected visceral myopathy or associated phenotypes included patients diagnosed with sporadic or familial forms of visceral myopathy, MMIHS, and congenital megacolon, megacystis syndrome (CMM). Patients with primary CIPO with a

demonstrable neuropathic origin were not included in this analysis. Patients with secondary CIPO were excluded. None of the subjects had prune belly syndrome, ophthalmoplegia or Barrett oesophagus, arterial aneurysms (seen with some *LMOD1*, *MYH11, MYLK* variants), peripheral neuropathy or papillary defects (seen in visceral neuropathy).

Written informed consent was obtained for participation in this study, which was approved by the Human Research Ethics Committee of the University of Western Australia in accordance with Australian National Health and Medical Research Council (NHMRC) guidelines and protocols. De-identification was completed by the assigning of a case number and removal of identifying information, with the cross reference held in a restricted and secure database maintained by PathWest and the WA Department of Health.

2.2 DNA sequencing

Initially, bi-directional Sanger sequencing of the eight coding exons (exons 2-9) of *ACTG2* (Accession number NM_001615.3) was performed on DNA isolated from peripheral blood. Sanger sequencing was performed on samples from family members, where available, to confirm co-segregation of the variant with disease.

In the probands for whom no *ACTG2* variant was identified, the coding region (three exons) of *LMOD1* (Accession number NM_012134.2) was Sanger sequenced. Screening of *MYH11* and *MYLK* was performed using the TruSight Cardio Sequencing Kit (Illumina) for nine of the genetically-unsolved probands. Panel sequencing was performed on a MiSeq (Illumina) in house in Diagnostic Genomics, PathWest. All sequencing and analysis was performed according to the National Association of Testing Authorities Australia (NATA) accredited protocols of the Department of Diagnostic Genomics. In the remaining proband, *MYH11* and *MYLK* were screened by exome sequencing (Ampliseq whole exome kit, Thermofisher Scientific) using an Ion Proton sequencer, as described previously(18). Average coverage of the exome was 72-fold with 90% of the exome covered >20-fold. Exome sequencing was performed by the Lotterywest State Biomedical Facility Genomics.

3. RESULTS

3.1 Patient cohort

The probands and their relatives (21 patients in total) from 17 families were analysed (Table 1). Fourteen probands had a diagnosis of primary CIPO with two of these having a familial disease and the rest appearing sporadically. Two probands had a diagnosis of congenital megacolon, megacystis (CMM) and one a diagnosis of megacystis-microcolon intestinal hypoperistalsis syndrome (MMIHS). Age of onset varied from *in utero* to 40 years.

Within the cohort, the most common features were abdominal distension (18/18 patients for whom this information was available), nausea and vomiting (15/17), abdominal pain (14/16) and malnutrition (13/15). Megacolon was seen in three cases and one case also had megaduodenum. Microcolon was recorded in a single case with prenatal MMIHS. Bowel resections, gastrostomies, colostomies and long-term catheterisation were common interventions (15 patients total). Six patients also required parenteral nutrition. Involvement of the urinary system (12/16) was common, with eight patients presenting with megacystis; other symptoms included urinary retention, large void volumes and recurrent urinary tract infections. Details of the cohort are summarised in Table 1.

3.2 *ACTG2* sequencing results.

Seven probands (10 patients) harboured variants in *ACTG2* (Table 1). All variants were absent from gnomAD and all involved substitutions at arginine residues. Four probands (three males, one female), with disease onset within the first year of life, had the previously reported recurrent variant(7), c.769C>T, p.Arg257Cys (rs587777387). In a male that presented at birth with pseudo-obstruction and megacystis a known variant(7), c.119G>A, p.Arg40His (rs587777386), was identified. In a proband with *in utero* detection of megacystis-microcolon-intestinal hypoperistalsis syndrome, a previously reported(7) p.Arg40Cys substitution (c.118C>T) (rs587777385) was identified. In four of six cases, family studies showed that the variants had arisen *de novo* in the proband. Parental DNA was not available for the other two families.

A proband with a strong family history (Family 1) of disease carried a c.443G>T, (p.Arg148Leu) variant (ClinVar submission SCV000207375.1 [Prof Croaker, The Canberra Hospital] (rs730880256)). Co-segregation studies showed that his affected son, brother and nephew also have this variant (Figure 1). The mother had a long history of gastrointestinal dysmotility including megacolon and atonic bowel requiring total parental nutrition, but DNA was not available.

Six of the seven *ACTG2* variant positive probands presented either *in utero* or within the first year of postnatal life. In the remaining family, Family 1, diagnosed with FVM, harbouring the p.Arg148Leu substitution, age of onset ranged from 20-40 years of age.

3.3 *ACTG2* variant negative probands

The nine *ACTG2* variant-negative probands had many features that overlapped those of the genetically resolved cases. Disease severity varied in this group, as it did in the *ACTG2* variant-positive group, with a range of intestinal and urinary symptoms requiring various interventions including bowel resections, parenteral nutrition and urinary catheterisation. Three (Probands 7, 9 and 15) of the ten variant-negative probands had a family history of disease, compared with one of seven of the variant-positive probands (Proband 1). Proband 7 had a similarly affected sibling. Proband 9 reported a strong family history of gastrointestinal problems (multiple affected children and grandchildren) and received a histopathological diagnosis of hollow visceral myopathy. The father and paternal grandmother of Proband 15 also suffer with gut dysmotility. The father had severe constipation as a child, and required a rectopexy at 21 years of age due to external rectal prolapse. Thus the likelihood of identifying an *ACTG2* variant does not appear to be higher in cases with a family history.

3.4 *LMOD1*, *MYH11* and *MYLK* sequencing results

LMOD1 was screened by Sanger sequencing in all ten *ACTG2* variant-negative probands but no variants were identified. *MYH11* and *MYLK* were screened using a next generation sequencing panel (n=9) or exome sequencing (n=1).

The only rare coding variant identified was a variant of unknown significance (c.1868, p.Ala623Gly; rs140688587) in *MYH11* in Proband 7 and her affected sibling. However

Sanger sequencing found that this variant is also present in the unaffected father. This substitution lies within the myosin motor head domain. It affects an amino acid residue conserved only to chicken, is glycine in frog and variable in other species. This variant was identified previously in a family with aortic aneurysm but did not segregate with disease and was thought to be benign(19). The variant is present in gnomAD in two of 277,210 alleles. The role of this rare variant in smooth muscle disorders therefore remains uncertain.

4. DISCUSSION

In this study we report current genetic diagnostic outcomes in an Australasian cohort of patients with clinical diagnoses of primary CIPO and who were suspected to have a visceral myopathy (degenerative leiomyopathy) or associated phenotype. We identified 10 patients from seven families with variants in *ACTG2*.

Previous studies have identified 18 different missense *ACTG2* variants in patients presenting with dominant visceral myopathy (OMIM 102545; (11)). There are four additional likely pathogenic variants or variants of unknown significance listed in ClinVar. All were submitted by the GeneDx service and there is no phenotypic information provided for these submissions. Experimentally, it has been shown that *ACTG2* variants (p.Arg40Cys, p.Arg63Gln, p.Arg148Ser, p.Arg178Cys/His/Leu) impair actin polymerisation and reduce cellular contractility(6, 20).

The variants are spread throughout *ACTG2*; however hotspots have been recognised, with Milunsky *et al.* reporting that 73% of the *ACTG2* families harbour variants altering residues Arg178 or Arg257 (33/49)(11). The p.Arg257Cys substitution was found in four of our seven *ACTG2* families. All of our mutation-positive cases harboured substitutions at Arg residues. The over-representation of substitutions affecting arginine residues of ACTG2 has been previously recognised(7).

Frequently, *ACTG2* variants occur *de novo* in the affected probands. Four of our seven *ACTG2* probands harboured *de novo* variants. In two families parental DNA was not available however *de novo* variants are suspected based on severe clinical presentations in the probands and absence of a phenotype in the parents. Of the 15 *ACTG2*-variant

positive families reported in Wrangler *et al*., 10 probands harboured *de novo* variants, in three families the variants were inherited and in two families the mode of inheritance was unknown(7). In Halim *et al*., eight of 11 probands with MMIHS were found to harbour *ACTG2* variants, in all cases the variants arose *de novo*(20). In another cohort study, four of 28 probands with CIPO harboured *ACTG2* variants, however all of the variant-positive cases presented with severe CIPO and megacystis(11).

Milunsky *et al*. (11) also reported that of all the published primary CIPO families/cases, 49 of 111 are *ACTG2* variant positive (44%). This is similar to the diagnostic rate in our cohort (7/16 families, 41%). It appears that *ACTG2* variants are a common cause of severe, early-onset disease, accounting for six of our seven *ACTG2* families and 11 of 16 MMIHS families described in Halim *et al*. and Morena *et al*.(10, 20). Six of the seven patients within our cohort that required parenteral nutrition harboured *ACTG2* variants.

Interestingly our dominant family with adult-onset disease (Family 1) harboured a variant (p.Arg148Leu) affecting the same amino acid as that of the original Finnish family (p.Arg148Ser; (3, 5)) in which onset was in adolescence or adulthood. Thus substitutions of Arg148 may result in a milder effect on protein function and hence a milder clinical presentation. A branch of this family has previously been reported with affected individuals harbouring the p.Arg148Leu substitution typically presenting with relatively late-onset disease and no involvement of the urinary system(21).

Monies *et al.* (12) recently reported a consanguineous family in which a child with severe visceral myopathy was homozygous for a nonsense variant (p.Arg168*) in *ACTG2*. Heterozygous parents and siblings were more mildly affected. An adult sibling with mild disease was also homozygous for the variant.

Variants in genes encoding all six actins result in human disease. Variants of *ACTA1*, encoding the skeletal muscle alpha-actin gene cause a range of skeletal muscle myopathies, including nemaline myopathy(22), variants of *ACTC1*, encoding cardiac alpha-actin, cause cardiomyopathies(23) and variants of *ACTA2* encoding smooth muscle alpha-actin cause thoracic aneurysms and aortic dissections and multi-system smooth muscle disorders(24). Variants of the ubiquitously-expressed beta- and gamma-

actins (encoded by *ACTB* and *ACTG1*) cause multi-system disorders(25). *ACTA1* variants account for 25% of nemaline myopathies, whilst *ACTA2* variants underlie 14% of thoracic aneurysms and aortic dissections(24). Thus, in comparison, variants of *ACTG2* underlying >40% of CIPO cases and represent a substantial contribution to the disease burden.

Some individuals with *ACTA2* variants present with multisystem smooth muscle disorder (aortic aneurysms, cerebrovascular disease, hypotonic bladder and gut hypoperistalsis)(26, 27). Thus normal expression of enteric gamma-actin in the gastrointestinal and urinary tract is not sufficient to escape disease. No cases of isolated CIPO have been reported due to *ACTA2* variants.

Despite the high contribution of *ACTG2* variants in CIPO and MMIHS, additional causative disease genes have been identified and others likely remain to be identified. Within our cohort, we did not identify pathogenic or likely pathogenic variants in the other CIPO/MMIHS genes: *LMOD1*, *MYH11* and *MYLK*. Next generation sequencing of well-phenotyped cohorts and/or large families with dominantly-inherited disease are likely to result in the identification of further causative genes for CIPO/MMIHS.

An accurate and timely genetic diagnosis of CIPO/MMIHS is critical for the optimal clinical management of patients with CIPO/MMIHS as it allows for appropriate interventions to avoid complications associated with secondary malnutrition and reduces the number of unnecessary tests (including biopsies and imaging). It also allows for pre-implantation or prenatal genetic diagnosis in subsequent pregnancies, where families may wish to avoid having an affected child.

The heterogeneity of terminology in current use is noted to be an ongoing challenge in investigating CIPO and its associated conditions. CIPO is an umbrella term for a broad clinical syndrome, associated with multiple subgroups, pathological descriptors, and specific genetic causes. Here, in the interests of a standardised but broad cohort, we have included all patients with primary CIPO who were suspected or known to have a visceral myopathy, as well as CIPO-associated conditions (CMM and MMIHS). Notably, patients with *ACTG2* variants regularly show urinary involvement which may contribute

to these disorders being described by different clinical syndromes or phenotypes in adult and paediatric studies. We anticipate that further studies into specific disease-causing variants and their clinicopathological correlation in primary CIPO and associated disorders, may eventually contribute to a simpler, accurate and standardized nomenclature.

In conclusion, given the relatively high contribution of *ACTG2* variants to disease and the frequency of *de novo* variants, *ACTG2* testing should be considered in patients presenting with hypoperistalsis.

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DISCLOSURE

No competing interests are declared.

AUTHOR CONTRIBUTIONS

Gianina Ravenscroft: GR study design, data collation, analysis and interpretation of data, manuscript preparation and review,
Stephen Pannell: SP data collation and analysis, manuscript preparation
Royston Ong: RO data collection and analysis
Hooi C. Ee; HCE conducted clinical assays and collected data
Fathimath Faiz: FF data collection and analysis
Lorna Marns: LM data collection and analysis
Himanshu Goel (HG) conducted clinical assays and collected data
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Elliot Sollis: ES data collection and analysis
Padma Sivadorai; PS data collection and analysis
Meredith Wilson: MW conducted clinical assays and collected data
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Scott Nightingale: SN conducted clinical assays and collected data
Mary-Louise Freckmann: M-LF conducted clinical assays and collected data
Edwin P Kirk: EPK conducted clinical assays and collected data
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Chamara Basnayake: CB conducted clinical assays and collected data
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Phillipa J Lamont: PL conducted clinical assays and collected data
David J Amor: DJA conducted clinical assays and collected data
Kristi Jones: KJ conducted clinical assays and collected data
Jaap Schilperoort: JS conducted clinical assays and collected data
Mark R Davis MRD contributed to study design
Nigel G Laing: NL study design, manuscript preparation and review
All authors approved the final version of the manuscript.

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FIGURE AND TABLE LEGENDS

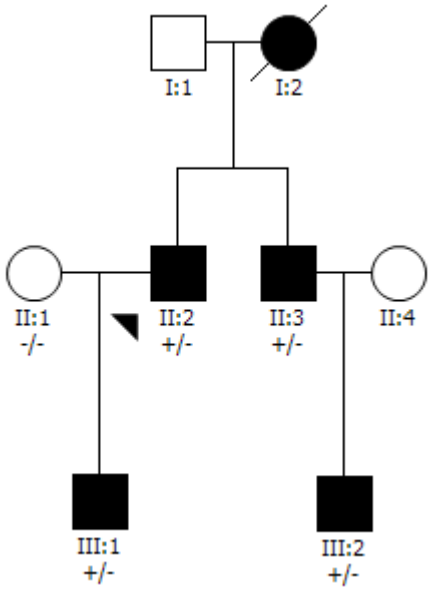
Figure 1: Pedigree showing segregation of the *ACTG2* variant in Family 1. Filled in symbols represent affected individuals. The proband is denoted by an arrow.

Table 1: Summary of the clinical and genetic findings in an Australasian CIPO cohort.

ID	Sex	Age at onset (y)	Age (y)	Referring diagnosis	*ACTG2* mutation	Inheritance	*ACTG2* mutation	Abdominal pain	Distension	Nausea/vomiting	Diarrhoea	Constipation	Malnutrition	Megacolon	Microcolon	Urinary symptoms	Megacystis	Urinary retention	Interventions	Notes
1; II;2	M	30	58	CIPO	c.443G>T p.R148L	Family history	c.443G>T p.R148L	+	+	+	+	-	+	NI	NI	+	+	-	PN, resection	
1; III:1	M	20	26	CIPO	c.443G>T p.R148L	inherited	c.443G>T p.R148L	+	+	+	+	-	+	NI	NI	-	NI	-		
1; II:3	M	40	53	CIPO	c.443G>T p.R148L	Family history	c.443G>T p.R148L	+	+	+	+	NI	NI	NI	NI	-	NI	-		
1; III:2	M	27	31	CIPO	c.443G>T p.R148L	inherited	c.443G>T p.R148L	+	+	+	+	+	+	NI	NI	NI	NI	NI	PN, resection	
2	M	I/U	(4)	CMM	c.119G>A p.R40H	NI	c.119G>A p.R40H	+	+	+	-	+	+	+	-	+	+	+	PN, laparotomy, ileostomy, vesicostomy	Megacystis I/U, cryptorchidism
3	F	0.15	3	CMM	c.769C>T p.R257C	*de novo*	c.769C>T p.R257C*	+	+	+	-	-	+	+	-	+	-	+	Catherisation, PN, ileostomy, laparotomy, resection,gastrostomy, jejunostomy	
4	M	0.75	15	CIPO	c.769C>T p.R257C	*de novo*	c.769C>T p.R257C*	NI	NI	NI	NI	NI	NI	NI	NI	+	NI	+		
5	M	0.33	15	CIPO	c.769C>T p.R257C	NI	c.769C>T p.R257C	NI	+	+	+	-	+	NI	NI	+	+	+	PN, Ileostomy	
6	M	0.5	11	CIPO	c.769C>T p.R257C	*de novo*	c.769C>T p.R257C*	+	+	+	-	+	+	NI	NI	+	+	+	Catheterisation, gastrostomy	
7a	F	5	28	CIPO		Family history		+	+	-	+	+	NI	NI	NI	-	-	-	NI	
7b	F	14	21	CIPO		Family history		-	+	+	-	-	+	NI	NI	-	-	-	NI	
8	F	24	35	CIPO		Isolated case		+	+	+	-	+	+	NI	NI	+	+	+	Subtotal colectomy, colostomy	Oedema, neuropathy
9	F	38	59	CIPO		Family History		NI	NI	+	+	+	+	NI	NI	NI	NI	NI	Subtotal colectomy	FH
10	F	Birth	29	CIPO		Isolated case		+	+	+		+	+	NI	NI	+	-	+	Total colectomy, ileoanal pouch	
11	F	Birth	7	CIPO		Isolated case		+	+	+	-	-	-	+	-	+	-	+	Colostomy	Megaduodenum
12	F	0.1	1	CIPO		Isolated case		+	+	+	+	-	-	NI	NI	NI	NI	NI	Colostomy	Billiary atresia
15	F	Birth	30	CIPO		Family		+	+	+	-	+	+	NI	NI	+	+	+	PN, colostomy, laparotomy, resection	Seizures,

						history													oedema, FH
16	F	2	8	CIPO		Isolated case		-	+	-	-	-	+	NI	NI	+	+	+	Fundoplication, button gastrostomy, jejunostomy	Seizures
17	F	NI		CIPO		Isolated case		NI	+	NI	NI	+	NI	NI	NI	NI	NI	NI	Catheterisation, vesicostomy	
18	F	NI		CIP		Isolated case		+	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	
19	F	I/U	TOP	MMIHS	c.118C>T p.R40C	*de novo*	c.118C>T p.R40C*	na	+	na	na	na	na	-	+	+	+	NI	na	TOP: 28 wg

Legend: CIPO: chronic intestinal pseudoobstruction; CMM: congenital megacolon, megacystis; FH: family history; I/U: *in utero*; MMIHS: megacystis-microcolon-intestinal hypoperistalsis syndrome; na: not applicable; NAD: no abnormality detected; NFSP: Nissen fundoplication sacral pacer; NI: no information; PN: parenteral nutrition; TOP: termination of pregnancy; VM: visceral myopathy; wg: weeks gestation; y: years; +: present; -: absent.. Age is given in years, numbers in parenthesis indicate the age at death.



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