Megacystis-microcolon-intestinal hypoperistalsis syndrome (MMIHS) is a rare disorder of enteric smooth muscle function affecting the intestine and bladder. Patients with this severe phenotype are dependent on total parenteral nutrition and urinary catheterization. The cause of this syndrome has remained a mystery since Berdon’s initial description in 1976. No genes have been clearly linked to MMIHS. We used whole-exome sequencing for gene discovery followed by targeted Sanger sequencing in a cohort of patients with MMIHS and intestinal pseudo-obstruction. We identified heterozygous ACTG2 missense variants in 15 unrelated subjects, ten being apparent de novo mutations. Ten unique variants were detected, of which six affected CpG dinucleotides and resulted in missense mutations at arginine residues, perhaps related to biased usage of CpG containing codons within actin genes. We also found some of the same heterozygous mutations that we observed as apparent de novo mutations in MMIHS segregating in families with intestinal pseudo-obstruction, suggesting that ACTG2 is responsible for a spectrum of smooth muscle disease. ACTG2 encodes $\gamma$2 enteric actin and is the first gene to be clearly associated with MMIHS, suggesting an important role for contractile proteins in enteric smooth muscle disease.
characterizing the clinical phenotype. MMIHS is part of a phenotypic spectrum that includes intestinal pseudo-obstruction [5] (OMIM 155310, 609629), hollow visceral myopathy [6,7] (OMIM 609629), pseudo-Hirschsprung disease [8], and irritable bowel syndrome [9]. Functional gastrointestinal obstruction is also frequently observed associated with other abnormalities such as prune-belly syndrome (OMIM 100100), external ophthalmoplegia (OMIM 277320), and Barrett esophagus (OMIM 611376). However, there is uncertainty about the extent to which locus heterogeneity and variation in expression underlie this clinical variability [9]. In addition, a number of single case reports have proposed an association of MMIHS with other disorders such as trisomy 18 [10], cardiomyopathymas [11], and deletion of 15q11.2 [12]. However, in these cases it is unclear whether these genetic disorders are related to MMIHS or are coincidental findings. Autosomal recessive inheritance of MMIHS (OMIM 249210) has been suggested in numerous cases based on the presence of two affected siblings [3,13,14], consanguinity [15] or both [16–19], but no genes have been identified to date, although in retrospect a report of a dominant mutation in the Chrnb2 gene and mice lacking both the Chrnb2 and Chrnb4 genes, respectively, displayed megacystis, failure of bladder strips to contract in response to nicotine, widely dilated ocular pupils, growth failure, and perinatal mortality [27,28]. These subunits are expressed in various sympathetic and parasympathetic ganglia, and lack of transmission at these ganglia could explain the lack of contraction of involuntary smooth muscle. A role for the α3 subunit was further suggested when reduced mRNA levels were measured by in situ hybridization, and reduced immunostaining for protein was possibly found in tissues from MMIHS patients [29]. However, antibodies against the neuronal nicotinic receptor subunits are notoriously unreliable [30] and a specific search for mutations in CHRNA3 and CHRNBD4 in many of the patients studied herein did not identify any potential disease-causing mutations [31].

**Results**

### Whole-Exome Sequencing in an MMIHS Cohort

Since the findings in mice harboring mutations in *Chrm3* or in *Chrm2* and *Chrm4* combined caused MMIHS-like phenotypes, we have conducted a study of MMIHS aimed at identifying the genetic cause. We collected samples from patients with MMIHS and related phenotypes, some of whom have been previously reported [31]. Our cohort of 34 families to date includes 27 DNA samples from probands including individuals diagnosed with MMIHS (20 probands) as well as intestinal pseudo-obstruction (4 probands), prune belly syndrome (2 probands), and hollow visceral myopathy (1 proband). Examples of radiologic findings are shown in Figure 1. Study recruitment has taken place over a period of 14 years.

We undertook whole-exome sequencing in 11 unrelated probands. The exome sequencing characteristics are summarized in Table 1. Of the 11 probands, eight were diagnosed with MMIHS and three diagnosed with intestinal pseudo-obstruction. We identified heterozygous missense variants in the *ACTG2* gene encoding γ2 enteric actin in six of the 11 individuals. We reasoned that *ACTG2* was an excellent candidate for MMIHS as a thin filament protein in the sarcorme involved in muscular contraction. We therefore undertook Sanger sequencing of all the exons and intron-exon boundaries of *ACTG2* in 16 additional probands in our cohort.

### De Novo and Inherited ACTG2 Mutations in the MMIHS Cohort

The results for all the heterozygous *ACTG2* variants in our cohort are summarized in Table 2. All of these variants were unique to our cohort, as none of the *ACTG2* variants found in our patients, were present neither within the 1000 Genomes project data (http://browser.1000genomes.org/index.html) nor within the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVSNHLBI/) nor within 1200 clinical samples analyzed in the BCM Hopkins Center for Mendelian Genomics (http://www.mendelian.org/) nor within 1200 clinical samples analyzed in the BCM clinical laboratory. We did identify a number of other novel heterozygous variants, but these were all distinct from the variants seen in our MMIHS cohort (Table S1). Within our group, 13 probands had mutations in *ACTG2* in comparison to 12 probands in the cohort without mutations in *ACTG2*. Of note, we observed ten apparently de novo events (Figure 2). We observed 6 novel C>T transition mutations at CpG dinucleotides affecting arginine amino acid residues including a recurrent mutation (c.769C>T; p.R257C) seen in 3 de novo cases (Fam4-1, Fam30-1, Fam25-1).
Clinical Characteristics of Smooth Muscle Disease Due to De Novo ACTG2 Mutations

The clinical characteristics of the patients with apparent de novo mutations are summarized in Table 3 and in Text S1. The age at the time of follow-up was from less than one year to 25 years. In the ten apparently de novo cases, seven patients were diagnosed with megacystis prenatally, and two of these underwent fetal surgery. The three individuals without megacystis were nonetheless dependent on catheterization of the bladder long-term. Prune-belly syndrome was observed in one of the cases (Fam16-1).

The gastrointestinal manifestations were similarly severe. Of the ten apparent de novo cases, seven had bilious vomiting as a neonate, and eight were diagnosed with intestinal malrotation. All ten patients had multiple abdominal surgeries (Table 4). Long-term dependence on TPN was a consistent feature, but did not extend throughout life for all the patients. Two patients had very intermittent TPN requirements, usually during surgical recovery. Another patient (Fam26-1) had an interval of improvement at age four years followed by reinitiation of TPN at six years.

Interestingly, of the ten apparent de novo patients, three reported partial but significant clinical improvement on cisapride, a serotonin 5-HT4 receptor agonist and gastroprokinetic agent (see Text S1). Recently this drug was removed from the market for cardiac side effects, but one patient continued on the drug as an FDA-approved case of compassionate use, and the two others indicated strong desires to remain on the drug despite the risks. Two other families found that the same drug did not have a significant effect.

Clinical outcomes in our cohort differ from the 19.7% survival rate reported in the literature [2]. Of the ten apparent de novo cases, nine were alive at the time of last follow-up; while one individual died at age 11 after multiple episodes of pancreatitis. One individual had undergone an intestinal transplant, and one individual was wait-listed for combined intestinal and liver transplantation.

Table 1. Exome analysis summary for six probands with MMIHS due to ACTG2 mutations.

| Subject | Total # unfiltered variants | Average Coverage | % at 10× coverage | % at 20× coverage | % at 40× coverage |
|---------|-----------------------------|------------------|-------------------|------------------|------------------|
| Fam4-1  | 129,171                     | 118              | 95.1              | 93.3             | 86.0             |
| Fam19-4 | 122,121                     | 80               | 93.1              | 89.4             | 75.2             |
| Fam25-1 | 146,726                     | 112              | 94.1              | 91.9             | 84.1             |
| Fam28-1 | 131,113                     | 132              | 92.5              | 89.5             | 81.9             |
| Fam29-1 | 132,740                     | 140              | 92.9              | 90.2             | 83.2             |
| Fam30-1 | 127,370                     | 135              | 93.0              | 90.3             | 83.1             |

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transplant. Of the nine surviving individuals, eight had ileostomies. The oldest survivor amongst the apparent \textit{de novo} cases was 25 years old, while the oldest previously reported case of MMIHS was 24 years \cite{2}. While this suggests improved survival in our cohort, we observed frequent abdominal surgery, and dependence on TPN and chronic catheterization, suggesting that improvements in supportive care over time rather than a milder phenotype associated with \textit{ACTG2} mutations are responsible for this improved survival.

\section*{Familial Disease Due to \textit{ACTG2} Mutations}

In addition to these apparent \textit{de novo} cases, five other probands were heterozygous for \textit{ACTG2} variants; in three of these cases the mutation was inherited from one of the parents. In one case the inheritance remains unknown as parental samples are not available. In an additional family (Fam19), the proband and an affected sibling both carry a heterozygous mutation that affects an alternate exon 4 (c.330C\textgreater{}A; p.F110L) of a predicted \textit{ACTG2} short isoform. The proband (Fam19-1) had multiple abdominal surgeries for obstruction and long-standing hypomotility. She has been on TPN intermittently since age 17 years, but has not required bladder catheterization. The sibling (Fam19-4) suffered years of intestinal symptoms and underwent an endoscopy suggesting gastroparesis. However she had not had any abdominal surgery. No parental clinical information was available. The mother does not carry this mutation, and a paternal sample is not available. The data from this family suggests but perhaps do not prove entirely that the alternative exon 4 which would result in a very short protein isoform is functionally important.

\section*{Predicted Functional Effect of the \textit{ACTG2} Mutations}

The mutations we observed extended from exon 2 to exon 7 of the transcript (Figure 3A). Alignment of all six human actin
proteins, which are highly conserved, revealed identity among all the amino acids in which we observed substitutions. All of these genes are implicated in human disease. De novo missense mutations in ACTB and ACTG1 underlie the brain malformation syndrome Baraitser-Winter (OMIM 243310) [32,33]. Mutations in ACTG2 are implicated in a range of cardiac phenotypes including cardiomyopathy (OMIM 613424) [34], cases of nemaline myopathy (OMIM 161800) are due to mutations in ACTA1 which can be dominantly or recessively inherited depending on the mutation [35], and ACTA2 mutations are associated with incompletely penetrant dominantly inherited aneurysm and dissections (OMIM 611800). We compared the ACTG2 mutations in our cohort to other exomes due to this pattern of codon usage, we surveyed paternal age differences to 18% of arginine residues genome wide altering an arginine codon (Figure 4A). We observed that the CGC codon encodes 33% of the arginine residues in the β-actin protein compared to 18% of arginine residues genome wide (Figure 4B) [36]. One explanation for the pattern of codon usage could relate to the expression of actin genes, as more highly expressed genes have been observed to have significantly skewed codon usage [37]. Given the presence of multiple CpG dinucleotides due to this pattern of codon usage, we surveyed paternal age in our de novo cases. We observed an average paternal age of 32.7 years amongst the families with de novo mutations with a standard deviation of 6.7 years which is not sufficient to conclude statistically whether these ten apparently de novo mutations may be associated with advanced paternal age.

Discussion

Identification of ACTG2 mutations underlying a significant proportion of MMIHS and intestinal pseudo-obstruction has significance for three major reasons. First, autosomal dominant rather than autosomal recessive mutations now are known to be present in the majority of families (15 of 26 probands reported in this study). Many cases in the literature as well as the Online Mendelian Inheritance in Man database suggest autosomal recessive inheritance. While other loci exhibiting recessive inheritance are possible, nearly half of our cases of MMIHS appear to follow a dominant or sporadic pattern of inheritance. While other loci exhibiting recessive inheritance are possible, nearly half of our cases of MMIHS appear to follow a dominant or sporadic pattern of inheritance. While other loci exhibiting recessive inheritance are possible, nearly half of our cases of MMIHS appear to follow a dominant or sporadic pattern of inheritance.

Second, the phenotypic spectrum for disease causing mutations in ACTG2 can now be relatively well defined. All of the apparent de novo cases had clear indications of severe smooth muscle disease with prenatal or neonatal onset, urinary catheterization, and

Table 2. Characteristics of the ACTG2 mutations in the MMIHS cohort.

| Subject | Position (hg19) Chr2 | cDNA change | Amino-acid change | CpG | Inheritance | Individuals with variant out of 1900
|---------|---------------------|-------------|------------------|-----|-------------|-------------------|
| Fam1-1  | 74141962 c.769C>T   | p.R257C     | +                |     | De novo     | 0                 |
| Fam11-1 | 74140693 c.533G>A   | p.R178H     | +                |     | Maternal    | 0                 |
| Fam12-1 | 74128450 c.119G>A   | p.R40H      | +                |     | De novo     | 0                 |
| Fam13-1 | 74141962 c.769C>T   | p.R257C     | +                |     | Maternal    | 0                 |
| Fam14-1 | 74129494 c.134T>C   | p.M45T      | –                |     | De novo     | 0                 |
| Fam16-1 | 74136215 c.412T>A   | p.Y134N     | –                |     | De novo     | 0                 |
| Fam17-1 | 74129547 c.187C>G   | p.R63G      | +                |     | Unknown     | 0                 |
| Fam19-4 | 74129825 c.330C>A   | p.F110L     | –                |     | Unknown     | 0                 |
| Fam25-1 | 74141962 c.769C>T   | p.R257C     | +                |     | De novo     | 0                 |
| Fam26-1 | 74128449 c.118C>T   | p.R40C      | +                |     | De novo     | 0                 |
| Fam28-1 | 74140753 c.593G>A   | p.G198D     | –                |     | De novo     | 0                 |
| Fam29-1 | 74140692 c.532C>T   | p.R178C     | +                |     | De novo     | 0                 |
| Fam30-1 | 74141962 c.769C>T   | p.R257C     | +                |     | De novo     | 0                 |
| Fam34-1 | 74128450 c.119G>A   | p.R40H      | +                |     | Paternal    | 0                 |
| Fam35-1 | 74140693 c.533G>A   | p.R178H     | +                |     | De novo     | 0                 |

*De novo mutations identified in 14 unrelated probands, six result from C>T transitions at CpG dinucleotides altering an arginine codon (Figure 4A). We observed that the CGC codon encodes 33% of the arginine residues in the β-actin protein compared to 18% of arginine residues genome wide.*

*Presence of the observed mutation in other exomes from the Baylor Center for Mendelian Genomics cohort.*

*Transcript Uc010fex.1.

*De novo cases had clear indications of severe smooth muscle disease with prenatal or neonatal onset, urinary catheterization, and
| Subject  | Fam4-1 | Fam12-1 | Fam14-1 | Fam16-1 | Fam25-1 | Fam26-1 | Fam28-1 | Fam29-1 | Fam30-1 | Fam35-1 |
|----------|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| ACTG2 Mutation | p.R257C | p.R40H | p.M45T | p.Y133N | p.R257C | p.R40C | p.G198D | p.R178C | p.R257C | p.R178H |
| Gender | M | M | F | M | M | M | F | F | M | F |
| Age in years | 11 | 16 | 18 | 25 | 13 | 16 | 3 | 3 | 1 | 6 |
| Paternal age at birth | 28 | 37 | 39 | 28 | 44 | 36 | 32 | 26 | 24 | 32 |
| Maternal age at birth | 31 | 33 | 35 | 26 | 36 | 33 | 31 | 32 | 26 | 28 |
| Megacystis | - | + | - | + | + | + | - | + | + | + |
| Fetal bladder diversion | - | + | - | + | - | - | + | + | - | - |
| Neonatal bilious emesis | + | - | + | - | + | - | + | + | - | - |
| Abdominal surgery/malrotation | + | - | + | - | + | - | + | + | - | + |
| Microcolon | - | - | + | - | + | - | + | + | - | - |
| Lifetime TPN dependence | + | - | - | - | + | + | + | + | + | + |
| Lifetime bladder catheterization | + | + | - | + | - | + | + | + | + | + |
| Motility treatment* | M (--) Cs(-) | Cs (+) | Cs (+) | Cs (+) | Cs (-) | Cs (-) | M (--) E (-) |
| Other medical conditions | Non-febrile seizures age 3 y | ADHD since age 6 y | Asthma, pectus excavatum, prune belly, cardiomyopathy | ADHD | Undescended testicle |

+ Feature present, − Feature absent, *M- Metaclopramide, Cs- Cisapride, E-Erythromycin, (+) responsive, (−) non-responsive.

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Table 4. Natural history of patients with MMIHS due to de novo ACTG2 mutations.

| Mutation | Ladd surgery/vesicostomy/jejunostomy, removal of bladder | Small bowel obstruction (SBO), ileostomy/1 y | Rectal biopsy/colostomy | Manometry with absent peristalsis | Functional outcome |
|---|---|---|---|---|---|
| p.R257C, p.R40C | neonate | | | | TNP dependent, cognition normal |
| p.R257C | | | | | |
| p.R40H | ileostomy/1 y | | | | |
| p.R178H | ileostomy | | | | |
| p.G198D | ileostomy/1 y | | | | |
| p.R178H | ileostomy/1 y | | | | |
| p.M45T | ileostomy/1 y | | | | |
| p.Y133N | ileostomy/1 y | | | | |
| p.R257C | ileostomy/1 y | | | | |
| p.R178H | ileostomy/1 y | | | | |
| p.G198D | ileostomy/1 y | | | | |
| p.R178H | ileostomy/1 y | | | | |
| p.M45T | ileostomy/1 y | | | | |
| p.Y133N | ileostomy/1 y | | | | |
| p.R257C | ileostomy/1 y | | | | |
| p.R178H | ileostomy/1 y | | | | |
| p.G198D | ileostomy/1 y | | | | |
| p.R178H | ileostomy/1 y | | | | |

Finaly our study effectively refocuses the study of MMIHS back to the contractile apparatus of the smooth muscle in an analogous way to how cardiomyopathy and myopathy tie to muscle contractile genes. Mendelian disorders of skeletal and cardiac muscle function have historically underscored the essential role of the sarcomere and its contractile apparatus in human health and disease [43,44]. Since Huxley formulated the sliding filament model, all muscle contraction has been understood as a product of the interaction of two polymers, the thin filament actins and thick filament myosins [45]. Mendelian disorders largely conform to Huxley’s fundamental insight as numerous disorders are now attributed to mutations in actins, myosins, and related proteins [46,47]. Sarcomere proteins had not been previously explored in MMIHS perhaps because the role of sarcomeric proteins in smooth muscle disease is less clear than in skeletal and cardiac disorders, and smooth muscle lacks the rigid alignment of the sarcomeres seen in cardiac and skeletal muscle. However, vascular smooth muscle disease has also been attributed to mutations in actin and myosin genes with the discovery of mutations in ACTA2 [48] and MIMI1 [49] in thoracic aortic aneurysms and dissections. There are also reports of a specific mutation in ACTA2 associated with vascular aneurysms and hypomotility of the gastrointestinal tract (OMIM 618384) [50] and also with prune belly sequence [51]. Additionally, as mentioned above, adult onset visceral myopathy was recently associated with a dominant mutation in the ACTG2 enteric actin gene in a Finnish family [20]. These findings provide the context for our data demonstrating a role for ACTG2 in MMIHS. While the mouse model for CHRNA3 generated promising insight into ganglion cell neurotransmission and smooth muscle function [27], the smooth muscle itself is clearly also involved in MMIHS. These results strongly suggest that there are other genes that are mutated in MMIHS, and candidate genes can be envisioned based on the combined data from mouse and human mutations. MMIHS can be considered the most severe Mendelian enteric smooth muscle myopathy.

After the submission of this manuscript, a report was published detailing the identification of de novo ACTG2 mutations by exome sequencing in two children with MMIHS [52].
**Materials and Methods**

**Study Subjects and Ethics Statement**

Informed consent was obtained prior to participation from all subjects or parents of recruited subjects under one of two Institutional Review Board approved protocols at Baylor College of Medicine.

**Clinical Evaluation**

Whenever possible, our clinicians assessed study subjects by direct history, physical examination, and family history analysis. In some cases, clinicians referred subjects from centers around the world, and in those cases clinical information in the form of chart records and notes from the referring physicians were reviewed. Interviews with these subjects were also conducted by telephone. Families were asked prenatal history, and dates and nature of abdominal surgeries. Whenever available, reports from prenatal ultrasound, operative reports, manometry, or radiologic studies were reviewed.

**Whole-Exome Capture and Sequencing**

Methods utilized for whole-exome sequencing have been previously described in detail [53]. In summary, 1 μg of genomic DNA was fragmented by sonication in a Covaris plate (Covaris, Inc. Woburn, MA). Genomic DNA samples were constructed into Illumina paired-end libraries as described [53]. Pre-capture libraries were pooled together and hybridized in solution to the BCM-HGSC CORE exome capture design [54] (52 Mb, NimbleGen). Captured DNA fragments were sequenced on an Illumina HiSeq 2000 platform producing 9–10 Gb per sample and achieving an average of 90% of the targeted exome bases covered to an minimal depth of 20× or greater.

**Data Analysis**

Produced sequence reads were mapped and aligned to the GRCh37 (hg19) human genome reference assembly using the HGSC Mercury analysis pipeline (http://www.tinyurl.com/HGSC-Mercury/). Variants were determined and called using the Atlas2 [55] suite to produce a variant call file (VCF) [56]. High-quality variants were annotated using an in-house developed suite of annotation tools [57].

**Sanger Sequencing**

Primers were designed to encompass all the exons and intron-exon boundaries of the ACTG2 gene using ExonPrimer (Tim Strom, http://ihg.gsf.de/ihg/ExonPrimer.html) and Primer3 [58]. Sanger reads were analyzed using LASERGENE Seqman software [59].

**Alignments and Analysis**

Multiple sequence alignments were performed using Clustal Omega [60] and depicted using Boxshade. Arginine codon usage

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**Figure 3. ACTG2 mutations affect conserved residues that are also targets for Mendelian disease.** A) Depiction of the mutations on the exons of the gene. Introns are not shown to scale. The mutations associated with MMIHS and intestinal pseudo-obstruction (orange) and those associated with intestinal pseudo-obstruction (green), including the previously reported mutation in one Finnish family are shown. A nonsense allele at position R63 was identified in our exome database associated with no clinical phenotype. The black, red, and blue lines under specific mutations highlight areas of multi-sequence alignment in boxes of corresponding colors in B. B) Comparison of the mutations in MMIHS/intestinal pseudo-obstruction with disease causing mutations in other actin genes.

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ACTG2 Mutations Cause MMIHS

A

Exon 2

| 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 |
|----|----|----|----|----|----|----|----|----|-----|
| M | C | S | E | T | T | A | L | Y | C | D | H | S | S | S | S | L | G | E |
| | | | | | | | | | | | | | | | | | | | | |

R40C/H

Exon 3

| 130 | 140 | 150 | 160 | 170 | 180 | 190 | 200 | 210 | 220 |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| A | T | C | T | C | A | G | T | G | C |
| | | | | | | | | | |

R63G

Exon 4

| 240 | 270 | 300 | 330 | 360 |
|-----|-----|-----|-----|-----|
| A | T | C | A | C |
| | | | | |

R148S

Exon 5

| 460 | 490 | 520 | 550 | 580 | 610 |
|-----|-----|-----|-----|-----|-----|
| G | I | V | L | D | S |
| | | | | | |

R178C/H

Exon 6

| 640 | 670 | 700 | 730 | 760 |
|-----|-----|-----|-----|-----|
| A | E | S | E | V |
| | | | | |

R257C/H

Exon 7

| 840 | 870 | 900 |
|-----|-----|-----|
| T | E | L |
| | | |

B

Arginine Codon Usage for Human Actin Genes

ACTG2

ACTG1

ACTC1

ACTB

ACTA2

ACTA1

Human genome

| CGU | CGC | CGA | CGG | AGA | AGG |
|-----|-----|-----|-----|-----|-----|
| 0   | 0   | 0   | 0   | 0   | 0   |

Frequency per 1000 codons
Figure 4. CpG dinucleotides within arginine codons are targets of de novo events in MMIHS. A) The coding exons are shown with translation for the ACTG2 gene. CpG dinucleotides are highlighted in red. Arginine residues in the protein are highlighted in green, and the mutations associated with ACTG2 smooth muscle disease are aligned above the sequence. B) The frequency of codon usage per 1000 codons for 6 arginine codons is shown. The human genome as a whole (bottom bar) is compared to all human actin genes including ACTG2 associated with translation for the

was determined using the Codon Usage Database and the countcodon program of Yuzakazu Nakamura.

**Supporting Information**

**Table S1** Novel variants in the ACTG2 gene within the Center for Mendelian Genomics data excluding the MMIHS cohort. (DOCX)

**Text S1** Clinical Case histories for cases of MMIHS due to ACTG2 mutations. A clinical narrative from birth to present is provided for each of the patients with the nature of the ACTG2 mutation. (DOCX)

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