The American Journal of Human Genetics, Volume *101*

Supplemental Data

Loss-of-Function Variants in *MYLK* Cause Recessive Megacystis Microcolon Intestinal Hypoperistalsis Syndrome

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Figure S1: AOH regions present in subject 1

Figure S2: Filtering criteria used to analyze the whole exome sequencing data of subject 1 and subject 3. High quality variants with a read depth ≥ 5 were retained for further analysis. An allele frequency cut-off of 1% in public databases (ExAC release 0.3, ESP6500SI-V2; 1000 Genomes Phase 3 release v5.20130502 and GoNL SNPs and Indels release 5) was considered for homozygous variants. Only variants predicted to affect splicing, nonsense variants, and coding and non-coding variants with a CADD score above 20 (CADD v1.3) were prioritized as likely deleterious. Subject 1 and subject 3 shared two genes with autosomal recessive variants. However, only *MYLK* had variants predicted to be deleterious and was expressed in fetal gut and bladder, the two major organs affected in MMIHS.

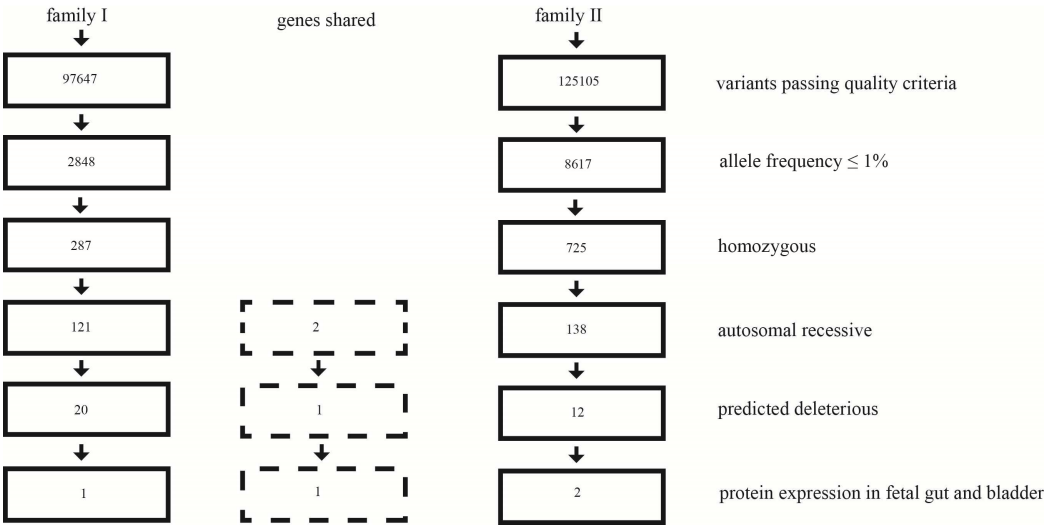

Figure S3: AOH regions present in subject 3

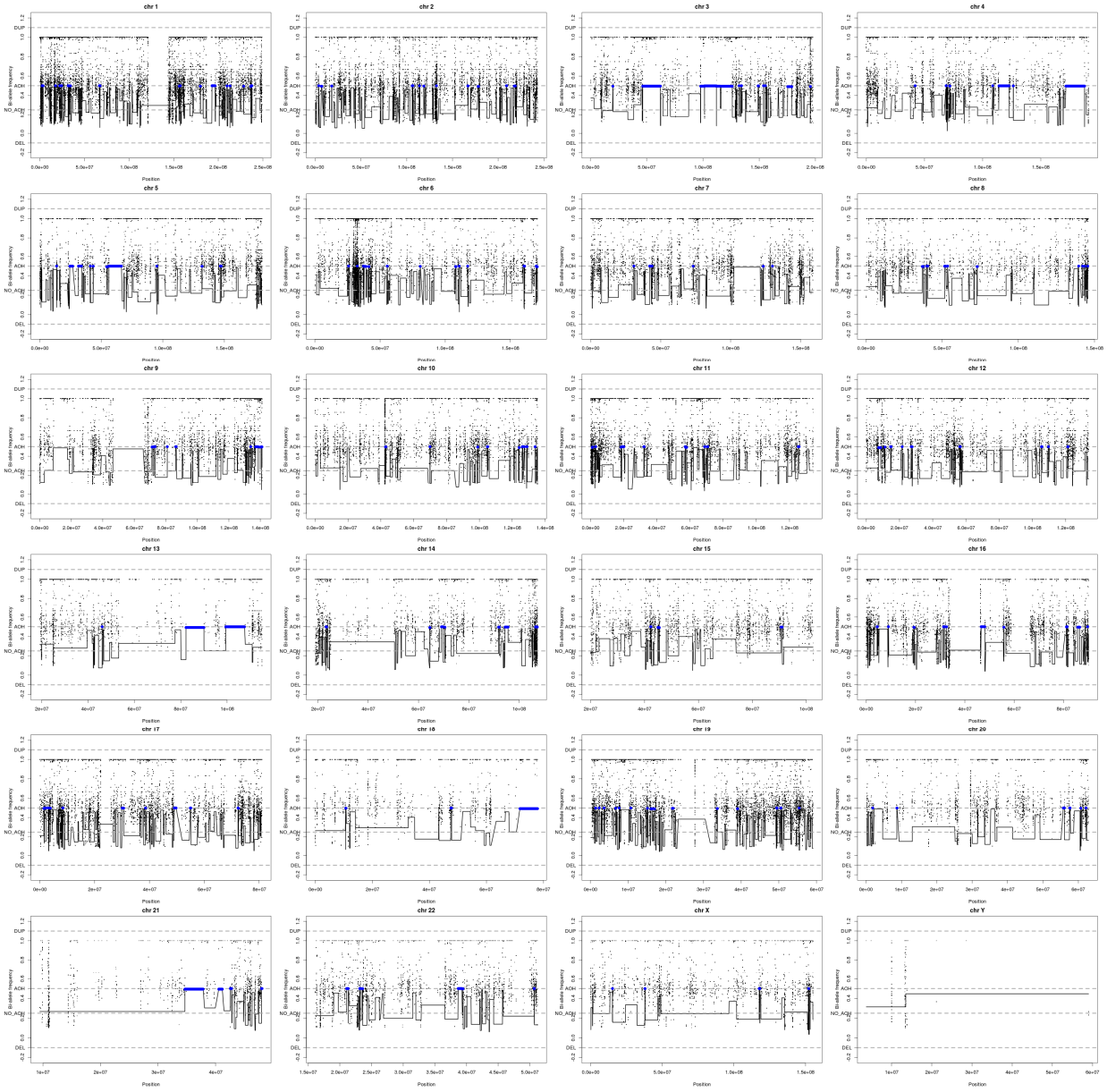

Figure S4: Schematic representation of *MYLK* with its 33 exons and its three *major* isoforms, with the variants identified in MMIHS affected individuals depicted.

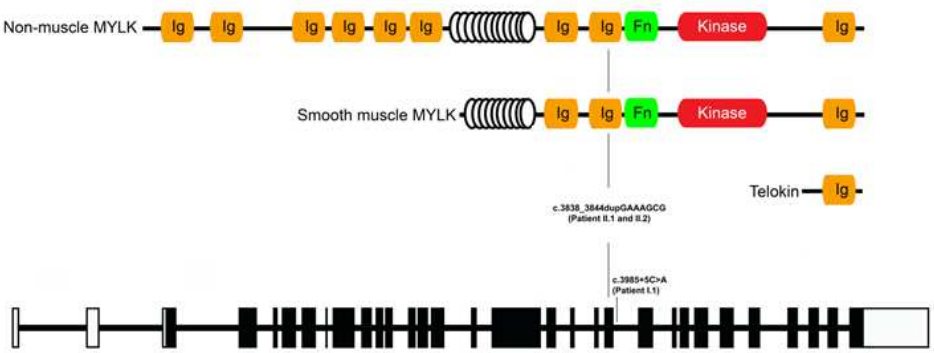

Figure S5: Schematic illustration of the molecular mechanisms required for SMC contraction and the effect of impaired *MYLK* expression on this mechanism. In the wild type situation, MYLK phosphorylates the regulatory light chain (RLC) of myosin, leading to its activation and subsequent interaction with actin filaments. As a consequence, SMCs contraction occurs. In the presence of any of the variants identified, *MYLK* expression is impaired, and phosphorylation of the RLC cannot occur. Therefore, myosin is unable to interact with actin filaments and SMC contraction is impaired.

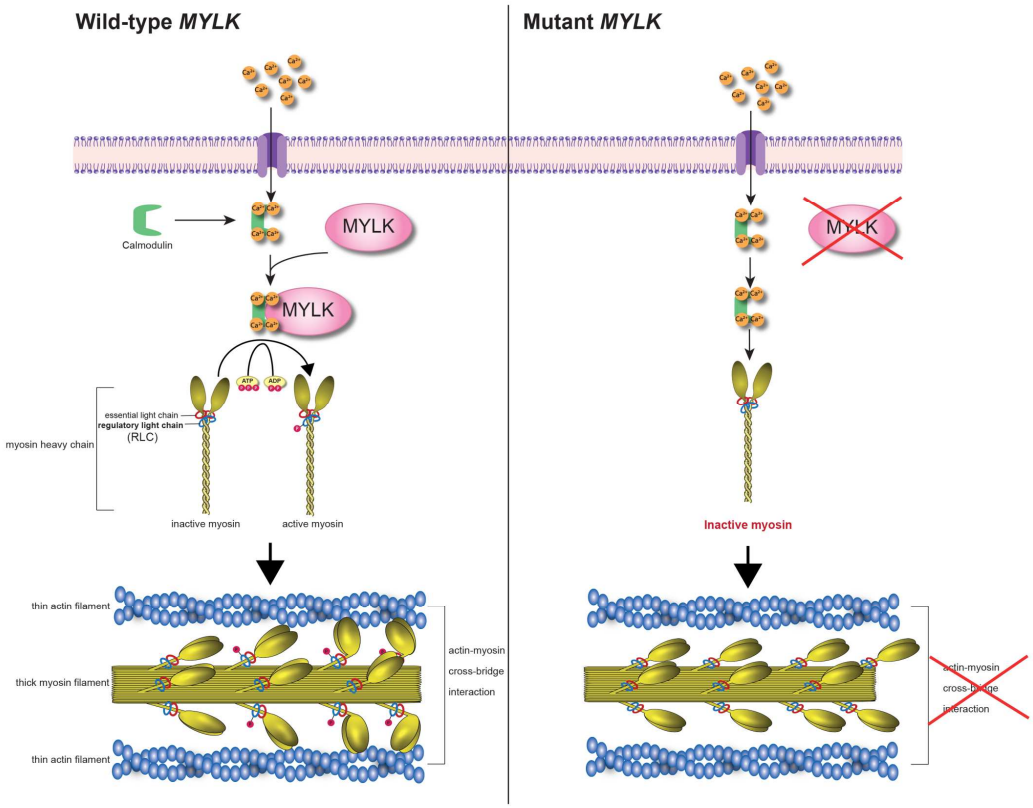

Table S1: ROH regions present in subject 1 (>1MB)

Chr.	ROH region	Size (kb)
1	chr1:242944438-244084838	1140400
1	chr1:145416055-147826789	2410734
1	chr1:150856155-165465569	14609414
1	chr1:70267535-120553638	50286103
2	chr2:193957725-194971610	1013885
2	chr2:228026937-231049859	3022922
2	chr2:132604119-139882966	7278847
2	chr2:231059532-240330999	9271467
3	chr3:120385537-121923035	1537498
3	chr3:121931699-124830052	2898353
4	chr4:87160439-91509561	4349122
4	chr4:77709496-86985942	9276446
5	chr5:92602370-93717798	1115428
5	chr5:41416674-42624527	1207853
5	chr5:87090359-88357858	1267499
5	chr5:179620442-180915260	1294818
5	chr5:1483897-8953381	7469484
6	chr6:15067080-19041846	3974766
6	chr6:19050234-25173355	6123121
7	chr7:122810117-123980215	1170098
7	chr7:101954468-103191686	1237218
7	chr7:40483744-43163338	2679594
7	chr7:29178667-40443341	11264674
8	chr8:0-2683108	2683108
8	chr8:46886735-119798778	72912043
9	chr9:70984372-84389822	13405450
11	chr11:54794237-56482051	1687814
11	chr11:79726393-91682741	11956348
12	chr12:42347636-71689131	29341495
13	chr13:23646794-24668110	1021316
15	chr15:36662007-54162140	17500133
16	chr16:68580385-73593216	5012831
17	chr17:44287381-45879796	1592415
17	chr17:41687610-44278785	2591175
18	chr18:334419-3513265	3178846
19	chr19:55569398-59128983	3559585
20	chr20:0-1613328	1613328
22	chr22:18922645-21800198	2877553

Table S2: ROH regions present in subject 3 (>1MB)

Chr.	ROH region	Size (kb)
1	Chr1:192999051-196461513	3462462
2	Chr2:23785517-24914125	1128608
2	Chr2:38809162-42181226	3372064
2	Chr2:47748292-48935849	1187557
2	Ch2:214228792-216256775	2027983
2	Chr2:217541582-218713924	1172342
3	Chr3:46480801-62229553	15748752
3	Chr3:97982936-101389864	3406928
3	Chr3:101443461-111936564	10493103
3	Chr3:111958510-115528917	3570407
3	Chr3:116163902-126070625	9906723
3	Chr3:175345143-178978718	3633575
4	Chr4:114028149-122683007	8654858
4	Chr4:171525788-187530313	16004525
5	Chr5:24510242-26915466	2405224
5	Chr5:31532429-34124590	2592161
5	Chr5:56271822-66480004	10208182
5	Chr5:112101793-114462589	2360796
5	Chr5:159776047-161128914	1352867
6	Chr6:37897839-39162227	1264388
6	Chr6:91278482-97096363	5817881
7	Chr7:64721546-66262309	1540763
7	Chr7:102330349-121081115	18750766
8	Chr8:136560952-139190957	2630005
9	Chr9:8518143-17579198	9061055
9	Chr9:46386688-65636192	19249504
9	Chr9:114985985-116130371	1144386
9	Chr9:136944465-138646881	1702416
9	Chr9:138656707-139944509	1287802
10	Chr10:19498278-20568524	1070246
10	Chr10:92813181-93841227	1028046
11	Chr11:18956194-20127046	1170852
11	Chr11:62064841-64525464	2460623
11	Chr11:65623347-67572698	1949351
12	Chr12:7463491-8688999	1225508
13	Chr13:82265231-90015578	7750347
13	Chr13:99449898-107516570	8066672
14	Chr14:94391699-95556747	1165048
16	Chr16:5257260-8740015	3482755
16	Chr16:46637746-48118036	1480290
17	Chr17:4997039-6014176	1017137
18	Chr18:71740503-77927028	6186525
19	Chr19:8151418-10229521	2078103
20	Chr20:2277020-4704957	2427937
20	Chr20:35263266-36375071	1111805
21	Chr21:34668747-37834258	3165511

Table S3: Primer sets used in this study

Primer	Sequence
*MYLK*F	TCAGGGAAGCTGGACTCTGG
*MYLK*R	CAGGGAGTCTGTGGGTTGC
MinigeneF	GCCCCCTTCCTTTCCTAGCC
MinigeneR	GCAGGGAGTCTGTGGGTTGC
SD6F	TCTGAGTCACCTGGACAACC
SA2R	GCTCACAAATACCACTGAGAT