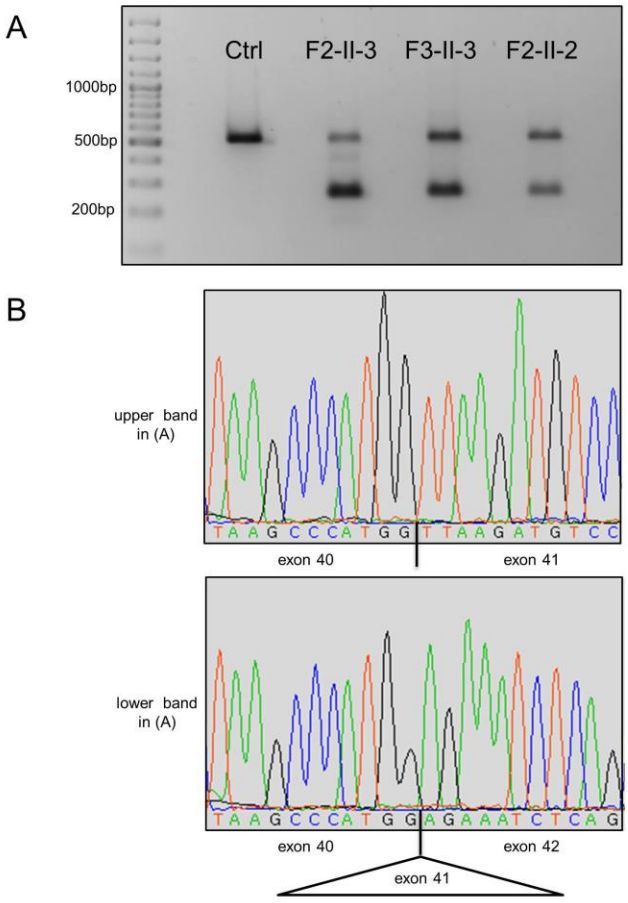
The American Journal of Human Genetics

Supplemental Data

Recessive Mutations in the α3 (VI) Collagen Gene *COL6A3* Cause Early-Onset Isolated Dystonia

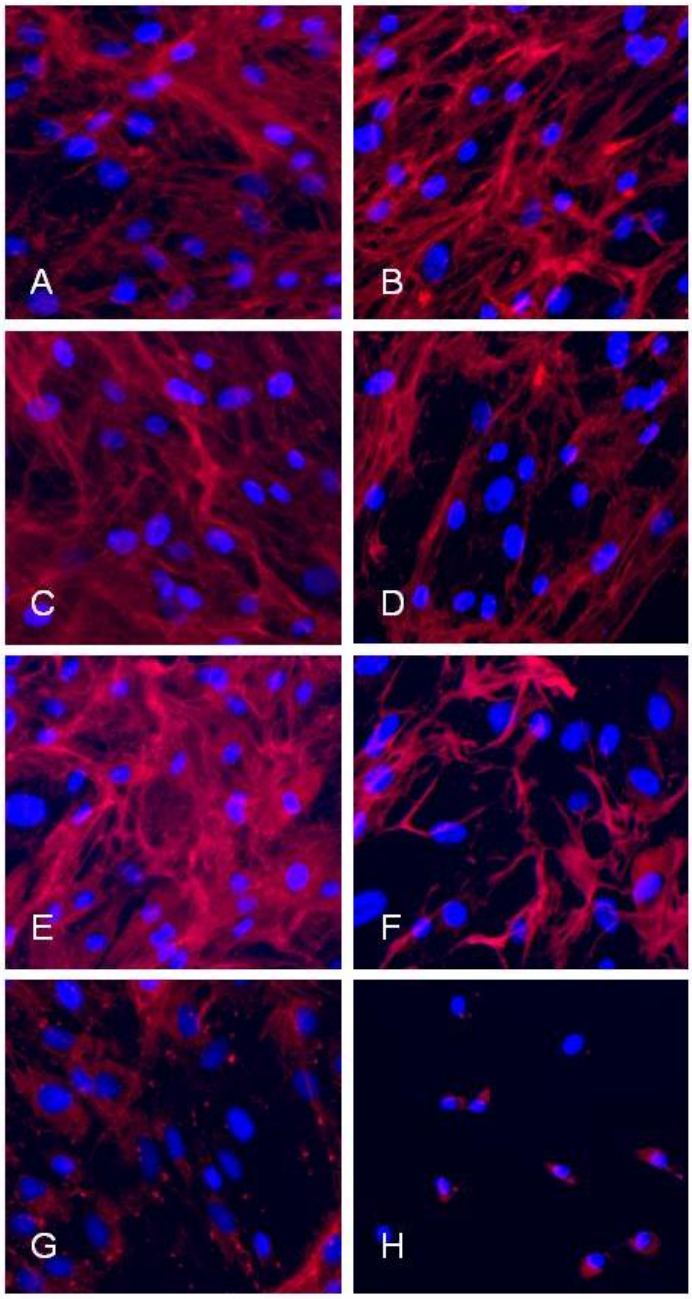
Michael Zech, Daniel D. Lam, Ludmila Francescatto, Barbara Schormair, Aaro V. Salminen, Angela Jochim, Thomas Wieland, Peter Lichtner, Annette Peters, Christian Gieger, Hanns Lochmüller, Tim M. Strom, Bernhard Haslinger, Nicholas Katsanis, and Juliane Winkelmann

Figure S1 The c.8966-1G>C mutation results in skipping of *COL6A3* exon 41



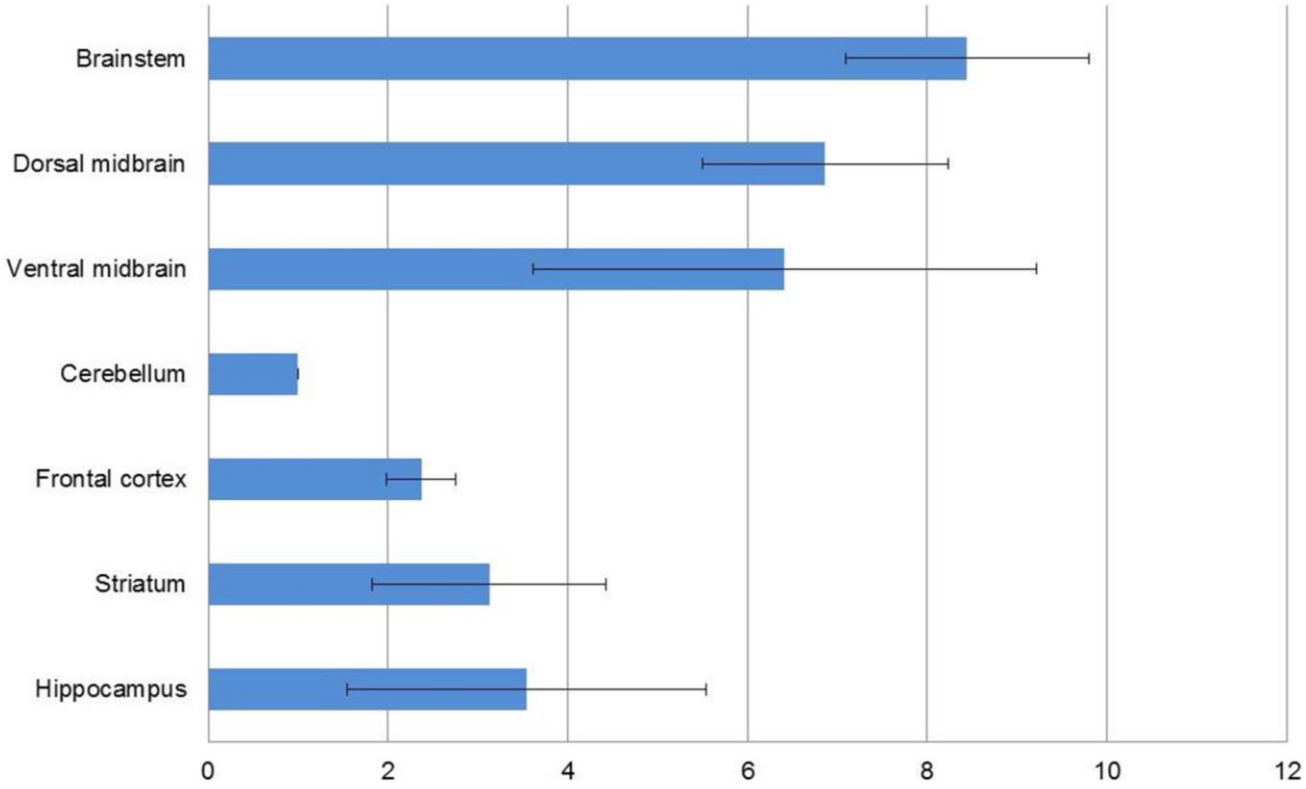
(A) cDNA gel electrophoresis showing an additional band in individuals carrying the canonical splice site variant c.8966-1G>C (individuals II-2 and II-3 of family F2, individual II-3 of family F3, Figure 1) but not in a normal control subject (Ctrl). (B) Sanger sequencing of cDNA corresponding to the upper band in (A) (522bp) reveals a normal transcript structure whereas the lower band in (A) (258bp) represents a transcript without exon 41. Primer pairs used are denoted in Table S8 and PCR conditions are available upon request.

Figure S2 Immunostaining of collagen VI in dermal fibroblast cultures



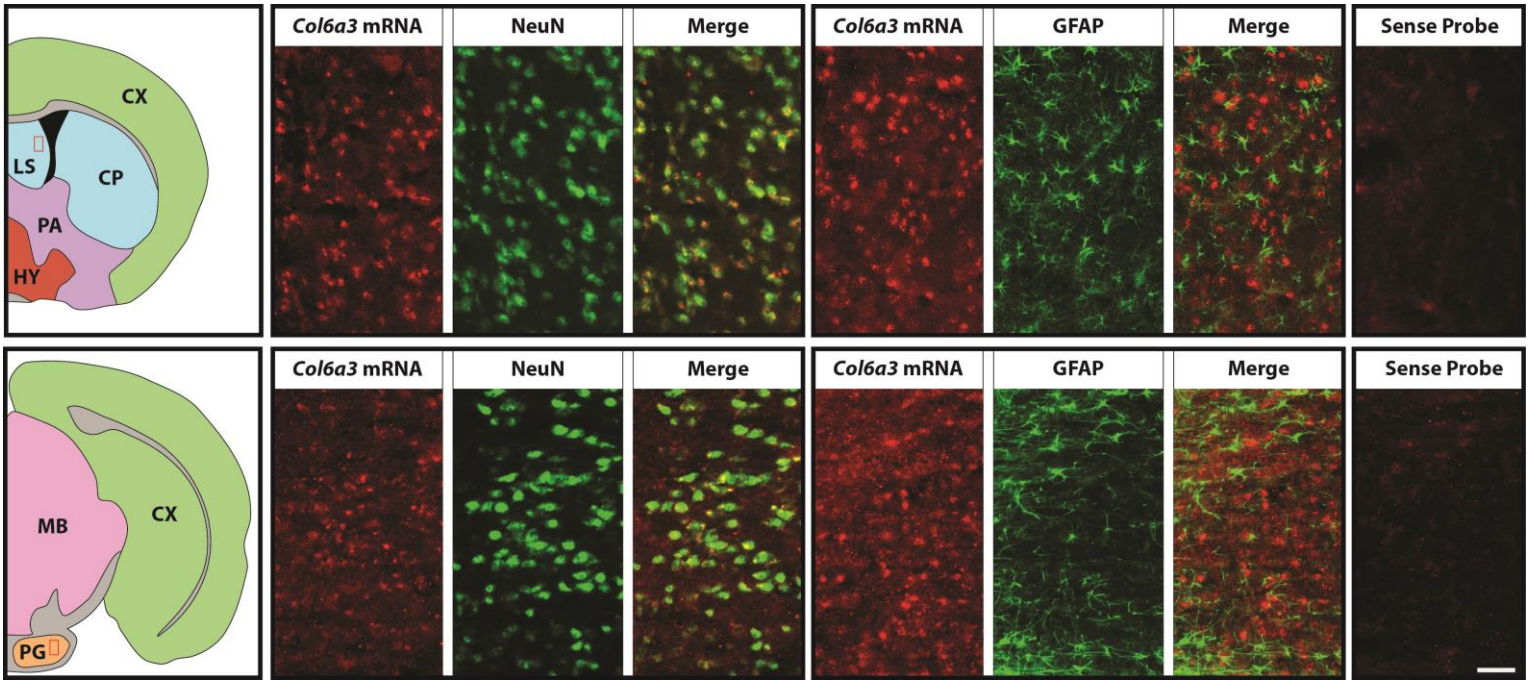
In a control subject (A), a healthy heterozygous *COL6A3* mutation carrier (individual F1-II-1, B), and isolated dystonia probands carrying compound heterozygous *COL6A3* mutations (individuals F1-III-1 [C], F1-III-3 [D], F2-II-3 [E], F3-II-3 [F]), collagen VI is highly expressed and effectively secreted into the extracellular space, where it forms beaded microfibrils (red matrix). In contrast, the protein is diminished in amount and displays an abnormal stippling pattern in an individual with Bethlem myopathy (G), and is extracellularly absent with some intracellular retention in an individual with Ullrich congenital muscular dystrophy (H). Cell nuclei were stained with DAPI (blue dots).

Figure S3 Expression of *Col6a3* in different mouse brain regions



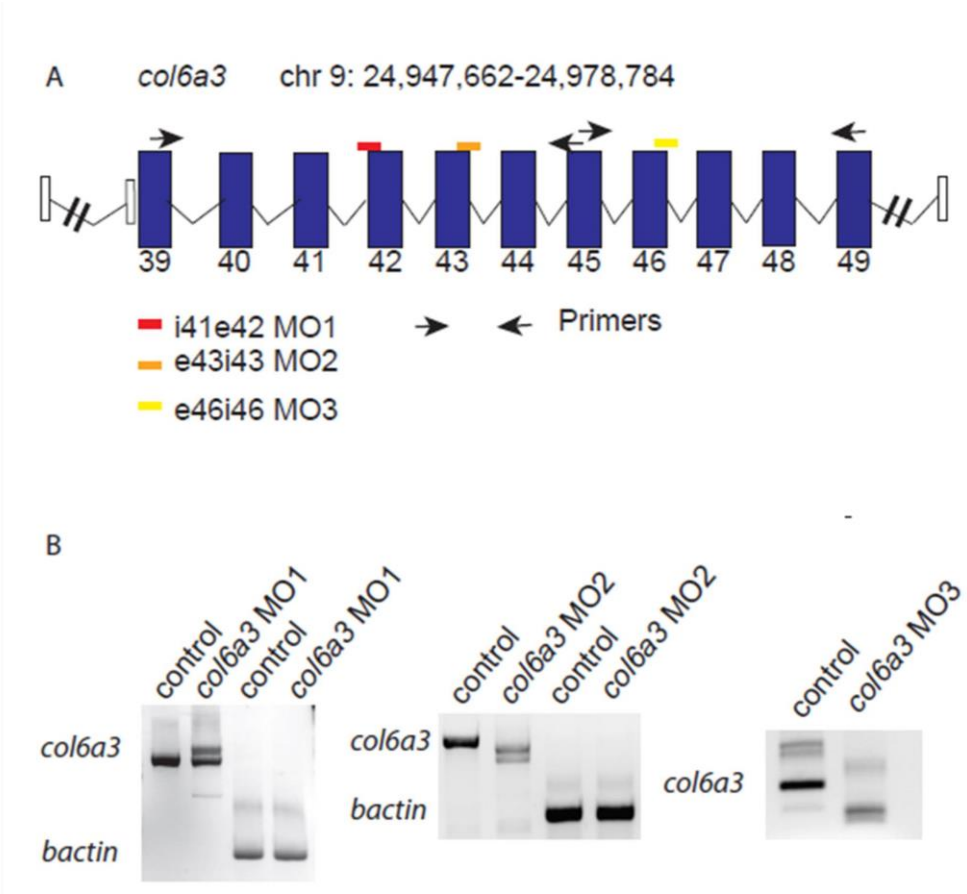
With QRT-PCR, relative expression of *Col6a3* was analyzed in seven different adult mouse brain regions. Relative mRNA expression levels are depicted as the fold change compared with the sample exhibiting the lowest expression. Average levels of three independent mice are shown. Error bars represent standard deviation.

Figure S4 *In situ* hybridization of coronal mouse brain sections with a *Col6a3*-specific antisense riboprobe and immunohistochemical detection of cell type markers



Simultaneous detection of *Col6a3* mRNA and neuronal cell marker NeuN (neuronal nuclear antigen), or astrocyte marker GFAP (glial fibrillary acidic protein) in the lateral septal nuclei (LS, top row) and the pontine grey (PG, bottom row). *Col6a3* mRNA co-localizes with NeuN-positive cells, but not with GFAP-positive cells (merged images). The sense probe indicates the specificity of the riboprobes used. CX=cortex, CP=caudate putamen, PA=pallidum, HY=hypothalamus, MB=midbrain. Scale bar represents 50 µm.

Figure S5 RT-PCR analysis of knockdown efficiency for splice-site targeted *col6a3* morpholinos



(A) Schematic of zebrafish *col6a3.* Splice blocking MOs designed against different *col6a3* exons: MO1 (red bar); MO1 (orange bar); MO3 (yellow bar). PCR primers (black arrows) designed against different exons were used to test MO efficiency. (B) In control embryos, there is a single amplicon, while in *col6a3* MO, there are two or three amplicons, demonstrating the efficiency of the MO used.

Table S1 Whole-exome sequencing statistics

Sample #	Reads	Mapped reads	Percent	Mapped sequence (Gb)	Target bases				
					>1x	>4x	>8x	>20x	Average coverage
F1-III-1	99,818,372	99,185,359	99.36	10.08	99.92	99.66	99.20	96.75	127.34
F1-III-3	96,609,280	96,056,442	99.43	9.76	99.80	99.54	99.08	96.78	125.85

Table S2 Shared sequence variants in affected individuals (F1-III-1, F1-III-3) identified by whole-exome sequencing

Synonymous variants	9691
NSVs	9857
NSVs with MAF < 0.3% (3,640 in-house exomes, NHLBI-ESP (EA), HapMap, 1000 Genomes)	102
≥ 2 NSVs with MAF < 0.3% / gene	3

NSVs = non-synonymous variants (missense, nonsense, stop-loss, splicing, insertions, deletions); MAF = minor allele frequency; NHLBI-ESP = National Heart, Lung, and Blood Institute-exome sequencing project; EA = European American.

Table S3 Candidate variants shared by individuals III-1 and III-3 of family F1 and Sanger sequencing validation

Gene	Genomic position (hg 19)	RefSeq transcript	Variation		dbSNP142	Mode	Segregation (Sanger sequencing validation)
			Nucleotide	Amino acid			
COL6A3	**chr2:238242176**	**NM_004369.3**	**c.9245C>G**	**p.Pro3082Arg**	**rs182976977**	**het**	**yes**
COL6A3	**chr2:238243370**	**NM_004369.3**	**c.9128G>A**	**p.Arg3043His**	**rs552651651**	**het**	**yes**
LTBP1	chr2:33482431	NM_206943.2	c.2248G>A	p.Val750Ile	rs80163321	het	no
LTBP1	chr2:33484669	NM_206943.2	c.2410C>T	p.Pro804Ser	rs149319598	het	no
SPNS3	chr17:4342998	NM_182538.4	c.245A>G	p.His82Arg	not found	het	no
SPNS3	chr17:4389800	NM_182538.4	c.1372G>A	p.Ala458Thr	rs140121510	het	no

Under a recessive model, only rare non-synonymous variants (NSVs) in the homozygous state or heterozygous but accompanied by a second rare heterozygous NSV in the same gene were retained. “Rare” indicates a frequency < 0.3% in 4,300 European American exomes of the NHLBI-exome sequencing project, 3,640 in-house exomes, HapMap, and the 1000 Genomes project. NSVs encompass missense, nonsense, stop-loss, and splice-site mutations, as well as insertions and deletions. The filtering approach yielded six different heterozygous NSVs in three genes whereas no homozygous changes were identified. Sanger sequencing validation within family F1 revealed that none but the two heterozygous *COL6A3* NSVs segregated with the disease status (highlighted in bold). Het = heterozygous.

Table S4 Demographics and clinical diagnoses of the German isolated dystonia cohort

Type of dystonia	No. (sex male/female)	Age at onset[a]	Age at sampling[a]	Family history positive no. (%)
Focal	257 (90/167)	47.2 ± 12.0 (5-81)	60.4 ± 15.9 (18-88)	30 (11.7)
Cervical dystonia	137 (52/85)	42.7 ± 13.6 (7-72)	56.9 ± 11.6 (28-84)	17 (12.4)
Blepharospasm	93 (29/64)	56.3 ± 14.8 (5-81)	68.3 ± 10.6 (26-88)	7 (7.5)
Writer`s cramp	9 (4/5)	32.2 ± 15.3 (14-62)	42.9 ± 19.7 (18-72)	1 (11.1)
Spasmodic dysphonia	17 (4/13)	42.0 ± 20.9 (6-79)	55.4 ± 17.6 (26-80)	5 (29.4)
Foot	1 (1/0)	43	48	0
Segmental	100 (25/75)	50.1 ± 14.1 (8-75)	65.6 ± 11.3 (37-87)	16 (16.0)
Generalized	10 (6/4)	22.6 ± 18.8 (7-57)	53.5 ± 12.7 (29-69)	2 (20)
Total	367 (121/246)	46.9 ± 17.7 (5-81)	61.6 ± 13.4 (18-88)	48 (13.1)

[a]Mean age in years ± standard deviation (range)

Table S5 Rare non-synonymous *COL6A3* exon 41/42 variants detected in 367 German isolated dystonia cases and 376 controls by Sanger sequencing

Exon[a]	Genomic position (hg19)	Variation nucleotide[a]	Variation amino acid[a]	Mutation type	dbSNP142	Frequency NHLBI-ESP (EA)	Carrier #	Sex/ Age at sampling	Dystonia distribution/ Age at onset	Additional rare NSV
41	chr2:238243533	c.8966-1G>C	p.Val2989_Lys3077del	canonical splice	not found	not found	Dys1	M/66	focal/24	p.Arg2501His (exon 36)
							Dys2	M/57	segmental/8	p.Ala2554Thr (exon 36)
41	chr2:238243520	c.8978G>A	p.Arg2993His	missense	rs201888442	T=4/C=8596	Ctrl1	F/83	control	none
41	chr2:238243370	c.9128G>A	p.Arg3043His	missense	rs552651651	not found	Dys3	M/53	focal/<10	none
41	chr2:238243350	c.9148G>A	p.Ala3050Thr	missense	rs114596320	not found	Dys4	F/60	focal/54	none
41	chr2:238243313	c.9185T>G	p.Leu3062Arg	missense	not found	not found	Dys5	F/69	segmental/51	none
42	chr2:238242176	c.9245C>G	p.Pro3082Arg	missense	rs182976977	C=8/G=8592	Dys6	F/40	segmental/37	none
							Dys7	F/62	segmental/ 53	none
							Ctrl2	F/82	control	none
							Ctrl3	M/66	control	none

The collagen VI α3 C4 domain (fibronectin type-III motif) encoding exons 41 and 42 were Sanger sequenced in 367 isolated dystonia cases from Germany and 376 ancestry-matched controls. When a rare non-synonymous variant (NSV) was identified, Sanger sequencing of the entire *COL6A3* coding region ensued to detect additional rare NSVs. "Rare" indicates a frequency < 0.3% in 4,300 European American (EA) exomes of the NHLBI-exome sequencing project (ESP) and 3,640 exomes in the in-house database. NSVs encompass missense, nonsense, stop-loss, and splice-site mutations, as well as insertions and deletions. Seven individuals with isolated dystonia and three control subjects carried rare NSVs in exons 41/42. The two individuals positive for the exon 41 canonical splice site mutation c.8966-1G>C (Dys1 and Dys2) carried a second rare *COL6A3* coding sequence alteration (a missense mutation in exon 36). Families of individuals Dys1 and Dys2 were further investigated (families F2 and F3 in Figure 1, individual Dys1 corresponds to individual F2-II-2 and individual Dys2 to individual F3-II-3 in Figure 1). F = female; M = male. [a]Numbering according to NCBI accessions NM_004369.3 and NP_004360.2.

Table S6 Rare non-synonymous *COL6A3* coding sequence variants detected in 360 German isolated dystonia cases and 373 controls without rare *COL6A3* exon 41/42 variants by high-resolution melting curve analysis

Exon[a]	Genomic position (hg19)	Variation nucleotide[a]	Variation amino acid[a]	Mutation type	dbSNP142	Frequency NHLBI-ESP (EA)	Carrier #	Sex/ Age at sampling	Dystonia distribution/ Age at onset	Additional rare NSV
3	chr2:238303650	c.289C>A	p.Arg97Ser	missense	not found	not found	Ctrl4	M/70	control	none
4	chr2:238296695-238296697	c.840_842 delAGT	p.Val281del	deletion	not found	not found	Dys8	M/86	segmental/48	none
4	chr2:238296513	c.1024G>A	p.Val342Met	missense	rs111402193	T=8/C=8592	Dys9	F/40	focal/39	none
							Dys10	F/39	focal/39	none
							Ctrl5	F/68	control	none
4	chr2:238296320	c.1217G>A	p.Arg406His	missense	not found	not found	Dys11	M/29	generalized/8	none
4	chr2:238296309	c.1228G>A	p.Asp410Asn	missense	rs35914491	T=6/C=8594	Ctrl6	F/81	control	none
5	chr2:238290075	c.1380C>G	p.Asn460Lys	missense	not found	not found	Ctrl7	F/72	control	none
6	chr2:238287473	c.2303G>A	p.Arg768His	missense	rs575412915	not found	Dys12	M/47	focal/44	none
7	chr2:238285960	c.2525T>C	p.Phe842Ser	missense	rs369930821	G=1/A=8599	Dys13	M/69	focal/48	none
7	chr2:238285819	c.2666G>A	p.Arg889His	missense	rs111295967	not found	Dys14	M/41	focal/40	none
7	chr2:238285811	c.2674G>A	p.Glu892Lys	missense	not found	not found	Ctrl8	F/75	control	none
7	chr2:238285477	c.3008G>A	p.Gly1003Glu	missense	not found	not found	Ctrl9	M/75	control	none
7	chr2:238285445	c.3040A>G	p.Lys1014Glu	missense	rs114284669	C=3/T=8597	Ctrl10	F/66	control	none
							Ctrl11	F/84	control	none
8	chr2:238283511	c.3223C>T	p.Arg1075Trp	missense	rs201962257	A=1/G=8599	Ctrl12	F/78	control	none
IVS9	chr2:238280984	c.3680-4 G>A		near-splice	rs376123972	T=3/C=8597	Dys15	F/75	focal/71	none
9	chr2:238280808	c.3852C>A	p.Phe1284Leu	missense	rs148561729	T=7/G=8593	Ctrl13	F/75	control	none
							Ctrl14	M/69	control	none
9	chr2:238280803	c.3857T>C	p.Leu1286Pro	missense	not found	not found	Dys16	F/65	focal/52	none
9	chr2:238280759	c.3901C>T	p.Arg1301Trp	missense	rs150430813	A=1/G=8599	Dys17	F/62	focal/14	none
9	chr2:238280737	c.3923G>A	p.Arg1308Gln	missense	not found	not found	Dys18	F/77	focal/63	none

9	chr2:238280557	c.4103C>T	p.Thr1368Met	missense	rs116505603	A=7/G=8593	Ctrl15	F/72	control	none
9	chr2:238280543	c.4117G>A	p.Ala1373Thr	missense	rs112181324	T=7/C=8593	Dys19	F/53	focal/37	none
							Dys20	F/74	segmental/65	none
							Ctrl16	F/82	control	none
							Ctrl17	M/85	control	none
9	chr2:238280539	c.4121A>T	p.Asp1374Val	missense	not found	not found	Dys21	F/70	focal/70	none
9	chr2:238280476	c.4184G>A	p.Arg1395Gln	missense	rs80272723	T=5/C=8595	Dys22	M/18	focal/14	none
							Ctrl18	M/74	control	none
11	chr2:238275489	c.5341A>G	p.Ile1781Val	missense	rs145447965	C=1/T=8599	Dys23	F/88	focal/70	none
12	chr2:238274346	c.5833G>A	p.Val1945Met	missense	not found	not found	Dys24	M/44	focal/35	none
IVS13	chr2:238273074	c.5839-3 C>T		near-splice	rs112825341	A=2/G=8598	Dys25	F/67	focal/44	none
14	chr2:238271954	c.6005A>T	p.Tyr2002Phe	missense	not found	not found	Dys26	M/40	focal/36	none
14	chr2:238271906	c.6053C>T	p.Ala2018Val	missense	rs200239695	A=1/G=8599	Dys27	M/55	focal/53	none
17	chr2:238268772	c.6241A>T	p.Thr2081Ser	missense	not found	not found	Dys28	F/78	segmental/54	none
21	chr2:238267204	c.6431A>T	p.Glu2144Val	missense	not found	not found	Ctrl19	F/81	control	none
23	chr2:238265998	c.6574G>A	p.Gly2192Arg	missense	not found	not found	Ctrl20	F/73	control	none
28	chr2:238258801	c.6868C>T	p.Arg2290Cys	missense	rs116608946	A=3/G=8597	Dys29	F/83	segmental/60	none
36	chr2:238253403	c.7258C>T	p.Arg2420Trp	missense	rs150165484	A=8/G=8592	Dys30	F/63	focal/62	none
37	chr2:238250725	c.7748C>T	p.Thr2583Met	missense	rs140021275	A=2/G=8598	Dys31	M/69	segmental/58	none
							Ctrl21	F/76	control	none
38	chr2:238249751	c.7808G>A	p.Arg2603Lys	missense	rs150554876	T=1/C=8599	Dys32	M/63	focal/53	none
38	chr2:238249631	c.7928C>T	p.Ala2643Val	missense	rs111595697	A=7/G=8593	Dys33	M/38	focal/30	none
38	chr2:238249550	c.8009C>T	p.Ala2670Val	missense	rs142851023	A=5/G=8595	Ctrl22	F/71	control	none
43	chr2:238234338	c.9358A>C	p.Thr3120Pro	missense	rs141050617	G=1/T=8599	Dys34	M/59	generalized/10	none
							Dys35	M/75	focal/67	none
43	chr2:238234219	c.9477A>C	p.Glu3159Asp	missense	not found	not found	Ctrl23	M/82	control	none

By means of high-resolution melting, *COL6A3* exons 1-40 and 43-44 were analyzed in 360 isolated dystonia cases from Germany and 373 ancestry-matched controls without rare non-synonymous variants (NSVs) in *COL6A3* exons 41/42. "Rare" indicates a frequency < 0.3% in 4,300 European American (EA) exomes of the NHLBI exome sequencing project (ESP) and 3,640 exomes in the in-house database. NSVs encompass missense, nonsense, stop-loss, and splice-site mutations, as well as insertions and deletions. Samples positive for more than one rare NSV were not identified. F = female; M = male. [a]Numbering according to NCBI accessions NM_004369.3 and NP_004360.2.

Table S7 Clinical, laboratory, imaging, and cellular investigations to exclude muscular pathology in families F1, F2, and F3

Individual	Status	Muscle weakness	Joint contractures	"Bethlem sign"	Joint hyperlaxity	Spinal rigidity	Skin abnormalities	Vital capacity in liters (respiratory involvement)	Serum creatine kinase level	Muscle MRI	Collagen VI immunolabeling in skin fibroblasts
Family 1 (F1)											
F1-II-1	unaffected	absent	absent	negative	absent	absent	absent	N/A	N/A	N/A	normal
F1-II-2	unaffected	absent	absent	negative	absent	absent	absent	N/A	N/A	N/A	normal
F1-III-1	affected	absent	absent	negative	absent	absent	absent	3.5 (absent)	normal	normal	normal
F1-III-2	unaffected	absent	absent	negative	absent	absent	absent	N/A	N/A	N/A	N/A
F1-III-3	affected	absent	absent	negative	absent	absent	absent	2.9 (absent)	normal	mild atrophic aspect likely due to physical inactivity	normal
Family 2 (F2)											
F2-II-2	affected	absent	absent	negative	absent	absent	absent	N/A	normal	N/A	normal
F2-II-3	affected	absent	absent	negative	absent	absent	absent	3.1 (absent)	normal	normal	normal
F2-III-1	unaffected	absent	absent	negative	absent	absent	absent	N/A	N/A	N/A	N/A
Family 3 (F3)											
F3-I-5	unaffected	absent	absent	negative	absent	absent	absent	N/A	N/A	N/A	N/A
F3-II-2	unaffected	absent	absent	negative	absent	absent	N/A	N/A	N/A	N/A	N/A
F3-II-3	affected	absent	absent	negative	absent	absent	absent	3.6 (absent)	normal	normal	normal

The phenotypic profile of *COL6A3*-related muscular disorders such as Ullrich congenital muscular dystrophy (UCMD) and Bethlem myopathy (BM) is characterized by pronounced proximal muscle weakness, joint contractures, joint hyperlaxity, spinal rigidity, skin abnormalities (e.g. follicular hyerkeratosis, abnormal scarring), and potential respiratory insufficiency. "Bethlem sign" defines a clinical test to aid in the recognition of subtle long finger flexor contractures. None of these clinical hallmarks were present in members of families F1, F2, and F3. Moreover, in all family members affected by isolated dystonia, serum creatinine kinase levels were in the normal range, and muscle MRI of pelvis and thigh did not show a pattern suggestive of myopathic or dystrophic changes. Finally, collagen VI immunolabeling in fibroblasts derived from isolated dystonia proband skin biopsies was not indicative of UCMD or BM, with the absence of typical alterations such as intracellular retention of collagen VI, diminished collagen VI secretion, or disorganization of collagen VI microfibrils. N/A = not available.

Table S8 *COL6A3* genomic and cDNA primer sequences

Genomic primers		
Exon	Forward Primer	Reverse Primer
ex2	ATTCCCTTTGCCCTGTTTTC	TATGTCAGTGCCTGATGTCTAAG
ex3_1	ACCAAATTTGTTTAGGAAGGC	CCATCCTTCGAGTGTCCATC
ex3_2	GAACCAATCAGACTGGAAAAGG	CAGTTGCCAAGCTCATCTCC
ex4_1	GGTTTCCAAAGAAACATGTCAG	CTACCACCCCGTAGCGAATC
ex4_2	CCTCGCCCTTGATTTCG	ATGCTGGTACCCACCTTACG
ex5_1	GGTGTCCTTTAAATGGAGTTAGC	CCAAAAGCTTAGGAATCCCC
ex5_2	GAAAATGAAGCCCCTGGAC	GTTCTTAAAGGCCCTGCCTG
ex6_1	TGTGCACGTGTTGTAGGCAC	CAGAAGCAGGAGCTGCG
ex6_2	TTCGGGCCTGAACACAG	TCAGACTGGCAAACTACCCTC
ex7_1	CGCTTTGGTTCCTGTTATGC	GGAACTGAAGCACTCCATCC
ex7_2	AGATCAAGACGGGCAAAGC	AGCCTACCCTCAACTCATGC
ex8_1	TTTGGACTTGGACATCCCTG	ATCATCCCCAGACCTGTCG
ex8_2	GCCCTGGAGTTTGTCCTG	CCAGACAAGTAGCCCCAGAC
ex9_1	GGTGGCTCATGAACGCTAAG	TTCCAGACGAGATGAGGACC
ex9_2	CAATGCCCTGGAGTACGTG	GAGTACCATGGCCTTTGAGC
ex10_1	CCTGGGTGAGGGCTACTTAC	ATCGTCCTGGGATTTTCCAC
ex10_2	CCACTGAACACTGGCAAGG	GAAGAAACAACCCAGAGAGAAG
ex11_1	TGTGTATTTATGTTCTGGGTCTGG	ACAAAGGCAATCTGAGGGAC
ex11_2	CAACACTAAGGTGGGCCTTG	CCAGGGACCAGACAGCTAAAG
ex12	AGAGTTCGTCTCCTCAGCCC	GAAGGGCTTCCTCCTTTCC
ex13	GCGAGTCTCTCAGTTTTCTATCAG	CAGTACACCCCGCCTCAC
ex14	ATTGCATTTTCCACATGCC	TCTTGAAATGCCAAAAGGTG
ex15	TTTATGATTTTCTGCTGGGC	GTCGGGCTTCTGACACC
ex16	GGGGACCACAGGGATTC	CCAATGGGTAAGGATCAAGG
ex17	GAGAAAGGGTGATGAAGGGC	CAGCATCTGGAGAAACTGC
ex18-20	ACAGCCCTTGGCCTCTTC	TTCCCTAAATGAAATGTTGATATTC
ex21	TTGCCTTCATGGTGAATGAG	TTCCCAACAACCCTCTTCC
ex22	CCTGACCATCCAGTCCAGTG	GCCGAGAAGTGTGTCCTTTC
ex23	TGTCTTGTGCAGCATTTCAG	TTGGGCAGATCTTATGGCAC
ex24	CTGAAAGAGCTCTAGGCCCC	TTTTCACTCCTGGGCTGG
ex25	GAGCTGACATTGACAGGAACC	CCGGATATAAATGCCACCC
ex26	GCTTCTCATGGAAAGTCACC	GAAACTCTCTCAGCCTGCTTG
ex27	AGAGACCAATTTGCCTGGAC	CTGACCAAAGAGGGAGAGGG
ex28	ATCACACTGTAAGCCAGCCC	GCACCTTCCAGCAAAGAGTC
ex29-30	GGCAGAGACGGTGGTTATG	GGACATACCAGAGCCATCCC
ex31	TCCTGGATGTCTTACCCCAC	AATTGTCAAAGCCTCAGCAG

ex32	TCCAACTATAGGAGCCTTAAATTC	AGGGACCACGTGTAAAGCAC
ex33-35	GGGCCAAAGCCTGTTTATTC	CTGTACTTACTACAAAAGGGAGGC
ex36	TTCTCCTAAGGAAAGGAAGCC	AACCTTCTCAGAGCCCCAG
ex37	TGTAGCTTTGATTTACTCCTCTGC	TCCCATGGATATTATGTTTTGC
ex38_1	AGCTGTGTATCCTGCTGTGG	CTTTCAAAGACATTCTCTATGGTG
ex38_2	CCCTGACTGACTATGGCTCC	CCTACACTTCTCCTGGCCC
ex39	TGTGATTCATTTAACTTGTTGACAG	CCCATAAAGTCAGGAGGTGG
ex40	TGTAGCTCATGGGGTTATGTC	ACCCTGGAGCAGGAAATGAG
ex41	CCTCTCTCGCTCATGCACTAC	TCCTTTGTGTCCTATTTGATACTCTAC
ex42	CTAAGGATTCCCAAGCCCTC	TTGGAATCAAGATGGGTATTTTC
ex43	CACTGGGAGCCGAGTAACAC	CAAGGTGACTTATTGACCTGAATC
ex44	CTTTTGGGTCAGTAATGTGGC	GTTGGCGATGGCTGACTC

cDNA primers		
	Forward Primer	Reverse Primer
exon 41 canonical splice site mutation	AAGCCAGCAGCTGTAAGACC	GCAAGTTCCTTCGTCTTTCG

Primer pairs used for direct sequencing of genomic DNA and cDNA, and for high-resolution melting curve analysis of *COL6A3 (*NM_004369.3).

Table S9 Expression data from five days post fertilization zebrafish heads in CPM (count per million reads)

Gene	Average (CPM)	STDEV
COL6A3	156.2	110.32
TOR1A	0	0
THAP1	5.2	1.37
GNAL	15.58	3.64
ANO3	1.91	1.23
GDAPH	874.62	373.72

The zebrafish RNAseq data can be retrieved from the NCBI Gene Expression Omibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE63191.