Mutation Profile of Collagen VI-Related Myopathy in Japan

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Research

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Abstract

Background: Collagen VI-related myopathy spans a clinical continuum from severe Ullrich congenital muscular dystrophy to milder Bethlem myopathy. This disease is caused by mutations in *COL6A1*, *COL6A2*, or *COL6A3*. Most reported mutations are *de novo*; therefore, to identify possible associated mutations, comprehensive large cohort studies are required for different ethnicities.

Methods: We retrospectively reviewed clinical information, muscle histology, and genetic analyses from 147 Japanese patients representing 130 families, whose samples were sent for diagnosis to the National Center of Neurology and Psychiatry between July 1979 and January 2020. Genetic analyses were conducted by gene-based resequencing, targeted panel resequencing, and whole exome sequencing, in combination with cDNA analysis.

Results: Of a total of 130 families with 1-5 members with collagen VI-related myopathy, 120 had mono-allelic and 10 had bi-allelic variants of *COL6A1*, *COL6A2*, or *COL6A3*. Among them, 60 variants were in *COL6A1*, 57 in *COL6A2*, and 23 in *COL6A3*, including 37 novel variants. Mono-allelic variants were classified into four groups: missense (69, 58%), splicing (40, 33%), small in-frame deletion (7, 6%), and large genomic deletion (4, 3%). Variants in the triple helical domains accounted for 88% (105/120) of all mono-allelic variants.

Conclusions: We report the mutation profile of a large set of Japanese cases of collagen VI-related myopathy. This dataset can be used as a reference to support genetic diagnosis and mutation-specific treatment.

Background

Collagen VI is an important component of the interstitium in skeletal muscles, and consists of three chains, alpha 1, 2, and 3, which are encoded by *COL6A1*, *COL6A2*, and *COL6A3* genes, respectively[1]. Mutations in *COL6A1*, *COL6A2*, or *COL6A3* cause a clinical continuum collectively called 'collagen VI-related myopathy'. At the more severe end of the continuum is Ullrich congenital muscular dystrophy (UCMD; OMIM 254090), and patients may have *de novo* mutations or show autosomal recessive inheritance. Bethlem myopathy (BM; OMIM 158810) is at the milder end, and patients mostly show autosomal dominant inheritance [2]. UCMD is the second-most common CMD in Japan [3]. In a study of the population in northern England, prevalence of UCMD was 0.13 cases per 100,000, whilst the prevalence of BM was 0.77 cases per 100,000 [4].

Collagen VI-related myopathy shows characteristic clinical phenotypes, which include proximal muscle weakness, skin and joint changes, scoliosis, and respiratory failure [1, 5, 6]. Muscle pathology encompasses variable histological changes including fiber size variation, an increased number of internal nuclei, and disproportionately prominent endomysial connective tissue considering the relative scarceness of necrotic and regenerating fibers [2, 7]. We have previously reported two patterns of collagen VI distribution in muscles among patients: completely deficient (CD) or deficient on the sarcolemma but with deposits in the interstitium (sarcolemma-specific collagen VI deficiency: SSCD) [3, 8].

The eventual diagnosis of this disease is made by genetic analysis. Before and in the era of next-generation sequencing (NGS), several studies have demonstrated a genetic spectrum in collagen VI-related myopathy, showing that a distribution of variants is common across several ethnic backgrounds [3, 6, 9-11]: the most common glycine substitution in the triple helical domain (THD), other missense variants, nonsense variants, splicing variants causing exon-skipping, small in-frame deletion/insertions, and small deletion/insertions causing a premature stop codon. Large genomic deletions spanning multiple exons are rare [5, 12-14]. Recently, a highly recurrent intronic mutation in *COL6A1* has been identified [15].

The aim of the present study was to elucidate the mutation profile of collagen VI-related myopathy in Japan by comprehensive genetic analysis including cDNA analysis, and to correlate the findings with immunostaining for collagen VI on muscle biopsies.

Methods

Clinical information

This retrospective cohort study was performed on patients seen at the NCNP, a major referral center for muscle disease in Japan, between July 1979 and January 2020. Frozen muscle and blood samples from patients were sent for diagnosis to the NCNP from all over Japan.

Clinically or pathologically suspected collagen VI-related myopathy with possible pathogenic variants in *COL6A1*, *COL6A2*, or *COL6A3* was identified in 147 affected individuals in 130 families. Patients with UCMD were classified into three categories according to phenotypic stratification as previously described [2, 24, 25].

This study was approved by the institutional review boards of the NCNP. All the human materials used in this study were obtained for diagnostic purposes. The patients or their parents provided written informed consent for use of the samples for research.

Muscle histology

Muscle biopsy samples for histological examination were frozen in isopentane cooled in liquid nitrogen. A set of routine histochemical analyses was performed for diagnosis. When the patients were suspected of having collagen VI-related myopathy or had elevated serum creatine kinase, immunohistochemistry was performed using standard procedures with an antibody against collagen type VI (VI-26, 1:1,000; MP Biomedicals, LLC, Irvine, CA) as previously described [3]. Immunofluorescence staining using standard procedures was performed with antibodies against collagen type VI (VI-26, 1:500; MP Biomedicals), PDGFR α (1:500, Cell Signaling Technology, Danvers MA), and laminin α 2 (4H8-2, 1:500; Santa Cruz, Dallas TX) [26].

Genetic analysis

Genomic DNA was isolated from peripheral blood lymphocytes or muscle specimens using standard techniques. All exons and their flanking intronic regions in *COL6A1*, *COL6A2*, and *COL6A3* were amplified and sequenced directly in 52 families using an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, Waltham, MA). Sixty-five families were analyzed using the target resequencing panel for muscular dystrophy because we developed a method for screening gene mutation in our laboratory since 2014 using Ion PGM NGS [27]. Thirteen families were analyzed by whole exome sequencing because they were initially suspected of having other types of muscular disease.

The splice site-creating variant Chr21:47,409,881 C>T in intron 11 of *COL6A1*, was manually screened by the Sanger method [15].

cDNA analysis

Total RNA was extracted from frozen muscle using a Total RNA Kit (Nippon Gene, Tokyo, Japan) and cDNA was synthesized with oligo (dT)₂₀ primer using SuperScript IV Reverse Transcriptase (Thermo Fisher Scientific, Waltham, MA) using standard techniques [8].

Identification of pathogenic variants

Novel pathogenic variants were identified using a previously described method [27] with modifications. Briefly, the likely pathogenic variants were defined according to the following criteria: (1) a glycine substitution in the THD; (2) causes exon skipping in the THD; (3) a large genomic deletion; (4) produces a nonsense codon or small insertion/deletion causing a premature stop codon in patients with bi-allelic variants; (5) a missense variant (except a glycine substitution or a substitution outside the THD). If outside the THD, the predicted amino acid substitution was a) predicted to be pathogenic by more than one *in silico* tool (PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>), MutationTaster (<http://www.mutationtaster.org/>), or CADD (<http://cadd.gs.washington.edu/>)), and/or b) co-segregated with the phenotype within a family. Missense variants were filtered with an allele frequency threshold of <0.01 in gnomAD (<https://gnomad.broadinstitute.org/>), NHLBI GO Exome Sequencing Project (<http://evs.gs.washington.edu/EVS/>), or the integrative Japanese Genome Variation Database (<https://ijgvd.megabank.tohoku.ac.jp>). The variants identified by target resequencing or whole exome sequencing were confirmed by Sanger sequencing.

Results

We identified pathogenic variants in a total of 130 families with collagen VI-related myopathy, which represented 1-5 members per family, seen at the National Center of Neurology and Psychiatry (NCNP) between July 1979 and January 2020, among them 120 families carried mono-allelic and 10 bi-allelic pathogenic variants (Table 1). One hundred and forty variants were identified, including 37 novel variants in 40 families, and these consisted of 60 allelic variants in *COL6A1*, 57 allelic variants in *COL6A2*, and 23 allelic variants in *COL6A3* (Fig. 1). In 94 families with a mono-allelic variant, this was sporadic without family history (94/130, 72%). Among the 37 novel variants, we identified 24 missense variants, six splicing variants, three small in-frame deletions, three large deletions, and one nonsense variant (Fig. 2).

Among the ten families with bi-allelic variants, in eight the variants were in *COL6A2*, while the other two each had variants in *COL6A1*, or in *COL6A3*. Six of these ten families had variants producing a premature termination codon or causing aberrant splicing, which leads to in-frame exon skipping in both alleles, and all had UCMD phenotypes. One of the ten families, #66, had a nonsense and a missense variant and also exhibited a UCMD phenotype. The affected individuals of the remaining three families had single nucleotide variants causing non-glycine substitutions and all showed BM phenotypes, although family #68 had a 26 bp-deletion causing a premature termination codon in one allele.

In the 120 families carrying a mono-allelic variant, the variants were as follows: missense (69, 58%), splicing (40, 33%), small in-frame deletion (7, 6%), and large deletion (4, 3%; Table 1). Variants in the THD accounted for 88% (105/120) and glycine substitution accounted for 48% (50/120). The variant c.868G>A (p.G290R) in *COL6A1* was found in eight families, while in 64 (53%) of the mono-allelic variant was unique. With respect to the genotype-phenotype correlation, the majority (82%, 87/105) of families having variants in the THD showed UCMD phenotypes, while the majority (93%, 14/15) of families harboring variants outside the THD showed milder phenotypes. It is important to note that all seven families showing the skipping of exon 14 in the THD of *COL6A1* had BM or mild UCMD phenotypes.

Three novel heterozygous multiple exon deletions were detected in four families (Fig. 3). The deletions spanned from exon 5 to exon 8 in *COL6A1* (Family #3 and #4), from exon 8 to exon 10 in *COL6A1* (Family #5), and from exon 8 to exon 10 in *COL6A2* (Family #87). All these large deletions were in-frame and distributed in the THD.

We performed immunostaining for collagen VI in muscle biopsies from 125 affected individuals in 123 families. In 115 patients with a mono-allelic variant, 91% (92/101) with the variant within and 71% (10/14) with the variant outside the THD showed SSCD. Even the biopsies from families harboring multiple exon deletions showed the typical SSCD staining pattern, suggesting dominant-negative effect of those variants (Fig. 4). Among the ten families having bi-allelic variants, five showed a CD pattern, while the five families carrying missense variant(s) showed a SSCD or a normal pattern. Observation at high magnification using immunofluorescence staining revealed trace amounts of extracellular collagen VI in the muscle biopsies of three families with CD (Family #64, #67, and #109), while collagen VI was retained within the mesenchymal cells in two families (#61 and #62; Fig. 4i–m).

We reviewed all available muscle imaging data (34 families). At least one of three typical findings in collagen VI-related myopathy (tigroid pattern in the vastus lateralis; target sign in the rectus femoris; a hyperintense rim between the soleus and gastrocnemius)[16] was seen in 85% (29/34) of the families. Among 29 families had mono-allelic variants in the THD, 86% (25/29) of these had typical imaging findings. Of the remaining five families, 3/4 (75%) with a variant outside the THD and the only family with a bi-allelic variant also showed typical imaging findings.

Discussion

We have elucidated the mutation profile of collagen VI-related myopathy in Japan (Table 1). Furthermore, we report 37 novel variants in 40 families, comprising 24 missense, six splicing, three small in-frame deletion, three large genomic deletion, and one nonsense. From the genetic information, we have established the mutation profile of the largest cohort at a single center as far as we are aware. The majority of the variants were mono-allelic (86%, 120/140), and 67% (94/140) of them were likely to be *de novo* because the parents of the patients were not affected, as has previously been described [6, 9, 10, 17, 18]. Therefore, our mutation profile may be useful as a reference for diverse ethnicities. Given that all cases with collagen VI-related myopathy in this cohort were sent to our center from hospitals in Japan, we calculated the occurrence of severe UCMD in Japan as 2.26 cases per year, which is an estimated incidence of 0.19 in 100,000 births, similar to that found for northern England (0.13/100,000) [4]. This is most likely because in both ethnicities the majority of variants are *de novo*.

Among the mono-allelic variants, 88% (105/120) were located in the THD. Mono-allelic variants in the THD must be primarily associated with the majority SSCD staining pattern (91%, 92/101) and UCMD phenotype (90%, 94/105), whilst mono-allelic variants outside the THD were also associated with SSCD (71%, 10/14) but a BM phenotype (93%, 14/15). In exceptional cases, genotypes cannot be associated with specific phenotypes, with some variants reported to cause both UCMD and BM phenotypes [9-11, 18]. In fact, in our cohort, the families with c.877G>A in *COL6A1*, c.856-2A>G in *COL6A2*, or c.943G>A in *COL6A2* showed a wide range of phenotypes from milder BM to severe UCMD, while conversely the variation in phenotypes of families with c.956A>G or c.1022G>A in *COL6A1* was quite narrow and on the border between UCMD and BM.

In addition, we found four heterozygous large deletions in families with UCMD phenotype. All the deletions were located in the N-terminal side of the cysteine residue important for the assembly of the collagen VI tetramer. This is in accordance with all the reported multiple exon deletions [12, 14, 19-22]. Intriguingly, the deletion in the region containing the cysteine residue caused relatively mild phenotypes in our cohort and in those of previous reports [6, 23]. Thus, collagen VI proteins with large genomic deletions, which have the deletions no more than amino acid residues may act in a dominant-negative fashion and show a UCMD phenotype.

In this study, we identified ten families having biallelic variants and five each of them showed CD and SSCD collagen VI staining patterns in muscles, respectively. We can presume that families with truncated variants in both alleles will be associated with CD and severe UCMD phenotypes, whilst those with missense variants or in-frame deletions at least in one allele will be associated with SSCD and milder BM phenotypes. In fact, three families with truncated variants in both alleles (CD) and five families with missense or in-frame deletion at least in one allele (SSCD) displayed compatible patterns with the aforementioned presumption, regardless of causative genes. Interestingly, the other two biallelic families had in-frame deletion(s) in one and in two alleles, but they showed CD and severe UCMD phenotypes. To explore the mechanism causing the loss of collagen VI in muscles in these families, we observed the trace of collagen VI remaining in their biopsied muscles. In muscles from patients with truncated variants in both alleles, collagen VI formed small deposits in the extracellular space, while in patients with an in-frame deletion in at least one allele, the collagen VI was retained within mesenchymal cells. Thus, in those cases with extracellular deposits visible, the truncated collagen VI molecules could form tetramers and be secreted, but the secreted collagen VI was unstable and degraded extracellularly. On the other hand, in the cases with a retained trace, the in-frame deleted molecules failed to make a tetramer and be secreted. Additional detailed molecular analyses are required to understand the precise mechanism.

Our results provide comprehensive information on mutation type, incidence, and their consequent effect, performing the role of a 'mutation catalog' that thereby replaces the multiple analyses. In the cases with splicing-site variants, intronic pseudoexon-creating variants, and genomic large deletions, which account for about one third of the total, cDNA analysis contributed to successfully identifying the variant. cDNA analysis was especially powerful in identifying intronic pathogenic variants (8%: 11/140) located outside of exon-intron borders and which led to cryptic splicing. Using a combination of genomic and transcript analyses with the collagen VI pathology observed in muscles, we were able to make a conclusive genetic diagnosis in 130/132 cases with suspected collagen VI myopathy clinicopathologically. However, multiple steps of analyses were required to reach the final genetic diagnosis. Because there are a large number of sporadic cases with a *de novo* collagen VI variant in this disease, our comprehensive mutation catalog together with mutation reports from other published cases may help genetic diagnosis in diverse ethnicities.

Conclusion

Our report provides a large mutation catalog of collagen VI-related myopathy in Japan, which can be used as a reference for genetic diagnosis and will also be helpful in mutation-specific therapy in the future. The majority of causal variants of collagen VI-related myopathy were mono-allelic *de novo*, and most of them were located in the THD and associated with SSCD and UCMD phenotypes.

Abbreviations

BM: Bethlem myopathy; CD: complete deficiency; SSCD: sarcolemma-specific collagen VI deficiency; THD: triple helical domain; UCMD: Ullrich congenital muscular dystrophy.

Declarations

Ethics approval and consent to participate

All clinical information and materials used in the present study were obtained for diagnostic purposes with written informed consent. The study was approved by the Ethics Committee of the National Center of Neurology and Psychiatry (NCNP).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contributions

MI and SN contributed to the conception and design of this study. MI, YS, TY, AI, MO, and SN analyzed and interpreted the data, and MI and SN wrote the manuscript. IN supervised the study. All authors read and approved the final manuscript.

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Tables

Table 1. Mutation profile of collagen VI-related myopathy

Family	Gene	Mono or Bi-allelic	Category	Nucleotide change	Protein change	Phenotype	COL6 IHC	Inheritance
1	COL6A1	BA	Missense	c.1879G>C homozygous	p.G627R	BM	Normal	Recessive
2-1 ^a	COL6A1	MA	Splicing	c.428+1G>T	p.Y77_G143del	BM	SSCD	Dominant
2-2 ^a	COL6A1	MA	Splicing	c.428+1G>T	p.Y77_G143del	BM	Normal	Dominant
3	COL6A1	MA	Large deletion	c.589-7_804+490del	p.E197_E285del	Mild UCMD	SSCD	Dominant
4	COL6A1	MA	Large deletion	c.589-7_804+490del	p.E197_E285del	Moderate Progressive UCMD	SSCD	<i>de novo</i>
5	COL6A1	MA	Large deletion	c.765_903+26del	p.P254_K301del	Moderate Progressive UCMD	SSCD	<i>de novo</i>
6	COL6A1	MA	Glycine substitution	c.806G>A	p.G269E	Moderate Progressive UCMD	SSCD	<i>de novo</i>
7	COL6A1	MA	Glycine substitution	c.833G>A	p.G278E	Moderate Progressive UCMD	SSCD	<i>de novo</i>
8	COL6A1	MA	Glycine substitution	c.841G>A	p.G281R	Mild UCMD	Normal	<i>de novo</i>
9	COL6A1	MA	Small deletion	c.845_847del	p.E282del	Moderate Progressive UCMD	SSCD	<i>de novo</i>
10	COL6A1	MA	Glycine substitution	c.849G>A	p.G284R	Early severe UCMD	SSCD	Dominant
11	COL6A1	MA	Glycine substitution	c.850G>A	p.G284R	Moderate Progressive UCMD	SSCD	<i>de novo</i>
12	COL6A1	MA	Glycine substitution	c.850G>A	p.G284R	Early severe UCMD	SSCD	<i>de novo</i>
13	COL6A1	MA	Glycine substitution	c.850G>A	p.G284R	Early severe UCMD	SSCD	<i>de novo</i>
14	COL6A1	MA	Glycine substitution	c.850G>A	p.G284R	Moderate Progressive UCMD	SSCD	<i>de novo</i>
15	COL6A1	MA	Glycine substitution	c.850G>A	p.G284R	Early severe UCMD	SSCD	<i>de novo</i>
16	COL6A1	MA	Glycine substitution	c.859G>C	p.G287R	Moderate Progressive UCMD	NA	<i>de novo</i>
17	COL6A1	MA	Glycine substitution	c.860G>A	p.G287E	Early severe UCMD	SSCD	<i>de novo</i>
18	COL6A1	MA	Glycine substitution	c.868G>A	p.G290R	Moderate Progressive UCMD	SSCD	<i>de novo</i>
19	COL6A1	MA	Glycine substitution	c.868G>A	p.G290R	Early severe UCMD	Normal	Dominant
20	COL6A1	MA	Glycine substitution	c.868G>A	p.G290R	Mild UCMD	SSCD	<i>de novo</i>
21	COL6A1	MA	Glycine substitution	c.868G>A	p.G290R	Moderate Progressive UCMD	SSCD	<i>de novo</i>
22	COL6A1	MA	Glycine substitution	c.868G>A	p.G290R	Mild UCMD	NA	<i>de novo</i>

23	COL6A1	MA	Glycine substitution	c.868G>A	p.G290R	Mild UCMD	SSCD	<i>de novo</i>
24	COL6A1	MA	Glycine substitution	c.868G>A	p.G290R	Moderate Progressive UCMD	SSCD	<i>de novo</i>
25	COL6A1	MA	Glycine substitution	c.868G>A	p.G290R	Mild UCMD	SSCD	<i>de novo</i>
26-1 ^b	COL6A1	MA	Glycine substitution	c.877G>A	p.G293R	Mild UCMD	NA	Dominan
26-2 ^b	COL6A1	MA	Glycine substitution	c.877G>A	p.G293R	Mild UCMD	NA	Dominan
26-3 ^b	COL6A1	MA	Glycine substitution	c.877G>A	p.G293R	Mild UCMD	NA	Dominan
27	COL6A1	MA	Glycine substitution	c.877G>A	p.G293R	Moderate Progressive UCMD	SSCD	<i>de novo</i>
28	COL6A1	MA	Glycine substitution	c.877G>A	p.G293R	Moderate Progressive UCMD	NA	<i>de novo</i>
29	COL6A1	MA	Glycine substitution	c.877G>A	p.G293R	Mild UCMD	SSCD	<i>de novo</i>
30-1 ^c	COL6A1	MA	Glycine substitution	c.877G>A	p.G293R	BM	NA	Dominan
30-2 ^c	COL6A1	MA	Glycine substitution	c.877G>A	p.G293R	BM	NA	Dominan
31	COL6A1	MA	Glycine substitution	c.895G>A	p.G299R	Mild UCMD	SSCD	<i>de novo</i>
32	COL6A1	MA	Glycine substitution	c.896G>A	p.G299E	Moderate Progressive UCMD	SSCD	<i>de novo</i>
33	COL6A1	MA	Splicing	c.930+189C>T	p.K310_G311insTRSTAPRRPLHLEGQGQPPRHPAK	Moderate Progressive UCMD	SSCD	<i>de novo</i>

Table 1. (continued)

Family	Gene	Mono or Bi-allelic	Category	Nucleotide change	Protein change	Phenotype	COL6 IHC	Inheritance
34	COL6A1	MA	Splicing	c.930+189C>T	p.K310_G311insTRSTAPRRPLHLEGQGQPPRHPAK	Mild UCMD	SSCD	<i>de novo</i>
35	COL6A1	MA	Splicing	c.930+189C>T	p.K310_G311insTRSTAPRRPLHLEGQGQPPRHPAK	Moderate Progressive UCMD	SSCD	<i>de novo</i>
36	COL6A1	MA	Splicing	c.930+189C>T	p.K310_G311insTRSTAPRRPLHLEGQGQPPRHPAK	Moderate Progressive UCMD	SSCD	<i>de novo</i>
37	COL6A1	MA	Splicing	c.930+189C>T	p.K310_G311insTRSTAPRRPLHLEGQGQPPRHPAK	Mild UCMD	SSCD	<i>de novo</i>
38	COL6A1	MA	Splicing	c.930+189C>T	p.K310_G311insTRSTAPRRPLHLEGQGQPPRHPAK	Moderate Progressive UCMD	SSCD	<i>de novo</i>
39	COL6A1	MA	Missense	c.956A>G	p.K319R	Mild UCMD	SSCD	<i>de novo</i>
40-1 ^d	COL6A1	MA	Missense	c.956A>G	p.K319R	BM	SSCD	Dominant
40-2 ^d	COL6A1	MA	Missense	c.956A>G	p.K319R	BM	NA	Dominant
41-1 ^e	COL6A1	MA	Missense	c.956A>G	p.K319R	BM	SSCD	Dominant
41-2 ^e	COL6A1	MA	Missense	c.956A>G	p.K319R	BM	NA	Dominant
41-3 ^e	COL6A1	MA	Missense	c.956A>G	p.K319R	BM	NA	Dominant
41-4 ^e	COL6A1	MA	Missense	c.956A>G	p.K319R	BM	NA	Dominant
41-5 ^e	COL6A1	MA	Missense	c.956A>G	p.K319R	BM	NA	Dominant
42	COL6A1	MA	Missense	c.957G>T	p.K319N	Moderate Progressive UCMD	SSCD	<i>de novo</i>
43	COL6A1	MA	Splicing	c.958-2A>T	p.G320_K334del	Early severe UCMD	SSCD	<i>de novo</i>
44	COL6A1	MA	Small deletion	c.958_966del	p.G320_E322del	Moderate Progressive UCMD	SSCD	<i>de novo</i>
45	COL6A1	MA	Small deletion	c.958_966del	p.G320_E322del	Early severe UCMD	SSCD	<i>de novo</i>
46	COL6A1	MA	Small deletion	c.958_966del	p.G320_E322del	Moderate Progressive UCMD	SSCD	<i>de novo</i>
47	COL6A1	MA	Small deletion	c.967_975del	p.K324_G326del	Early severe UCMD	SSCD	<i>de novo</i>
48	COL6A1	MA	Splicing	c.1003-1G>A	p.G335_D352del	BM	SSCD	<i>de novo</i>
49	COL6A1	MA	Glycine substitution	c.1022G>A	p.G341D	Mild UCMD	SSCD	<i>de novo</i>
50-1 ^f	COL6A1	MA	Glycine substitution	c.1022G>A	p.G341D	BM	SSCD	Dominant
50-2 ^f	COL6A1	MA	Glycine substitution	c.1022G>A	p.G341D	BM	NA	Dominant
51	COL6A1	MA	Glycine substitution	c.1022G>T	p.G341V	BM	Normal	Dominant
52	COL6A1	MA	Splicing	c.1056+1G>A	p.G335_D352del	Mild UCMD	SSCD	<i>de novo</i>
53	COL6A1	MA	Splicing	c.1056+1G>A	p.G335_D352del	Mild UCMD	SSCD	<i>de novo</i>
54	COL6A1	MA	Splicing	c.1056+1G>A	p.G335_D352del	Mild UCMD	SSCD	<i>de novo</i>
55-1 ^g	COL6A1	MA	Splicing	c.1056+1G>A	p.G335_D352del	BM	Normal	Dominant
55-2 ^g	COL6A1	MA	Splicing	c.1056+1G>A	p.G335_D352del	BM	NA	Dominant
56-1 ^h	COL6A1	MA	Splicing	c.1056+1G>A	p.G335_D352del	BM	SSCD	Dominant

56-2 ^h	COL6A1	MA	Splicing	c.1056+1G>A	p.G335_D352del	BM	NA	Dominant
57	COL6A1	MA	Splicing	c.1056+3A>C	p.G335_D352del	BM	SSCD	<i>de novo</i>
58	COL6A1	MA	Glycine substitution	c.1138G>A	p.G380R	Early severe UCMD	SSCD	<i>de novo</i>

Table 1. (continued)

Family	Gene	Mono or Bi-allelic	Category	Nucleotide change	Protein change	Phenotype	COL6 IHC	Inheritance	Report
59	COL6A1	MA	Glycine substitution	c.1255G>A	p.G419S	BM	Normal	Dominant	Novel
60	COL6A1	MA	Splicing	c.1461+4A>G	p.G467_E487del	BM	Normal	<i>de novo</i>	[21]
61	COL6A2	BA	Splicing Splicing	c.1270-1G>C c.1771-3C>G	p.G424_K444del p.G591fs	Early severe UCMD	CD	Recessive	[3]
62	COL6A2	BA	Splicing	c.1572+1G>C homozygous	p.G508_P524del	Moderate Progressive UCMD	CD	Recessive	[3]
63	COL6A2	BA	Splicing Small deletion	c.1770+5G>A c.2267_2272del	p.G562_T573del p.A756_I757del	Moderate Progressive UCMD	SSCD	Recessive	[8] [8]
64	COL6A2	BA	Splicing Small deletion	c.1771-2A>T c.2279_2280del	p.G591fs p.D761fs	Moderate Progressive UCMD	CD	Recessive	[3]
65-1 ⁱ	COL6A2	BA	Missense Missense	c.2093C>T c.2927T>C	p.A698V p.L976S	BM	SSCD	Recessive	Novel Novel
65-2 ⁱ	COL6A2	BA	Missense Missense	c.2093C>T c.2927T>C	p.A698V p.L976S	BM	NA	Recessive	Novel Novel
66	COL6A2	BA	PTC Missense	c.2386A>T c.2584C>T	p.K796X p.R862W	Moderate Progressive UCMD	SSCD	Recessive	Novel, [34]
67	COL6A2	BA	Small deletion	c.2678_2700del homozygous	p.P893fs	Moderate Progressive UCMD	CD	Recessive	[3]
68	COL6A2	BA	Missense Small deletion	c.2488C>T c.1487_1512del	p.R830W p.R498fs	BM	SSCD	Recessive	[35], [36]
69	COL6A2	MA	Missense	c.167G>A	p.S56N	BM	SSCD	<i>de novo</i>	Novel
70	COL6A2	MA	Missense	c.565G>A	p.A189T	BM	Normal	<i>de novo</i>	Novel
71	COL6A2	MA	Splicing	c.736-1G>A	p.C246_K267del	Moderate Progressive UCMD	SSCD	<i>de novo</i>	[37]
72	COL6A2	MA	Glycine substitution	c.785G>T	p.G262V	BM	SSCD	Dominant	Novel
73	COL6A2	MA	Splicing	c.801+1G>T	p.C246_K267del	Moderate Progressive UCMD	SSCD	<i>de novo</i>	[11]
74	COL6A2	MA	Splicing	c.801+2T>C	p.C246_K267del	Moderate Progressive UCMD	SSCD	<i>de novo</i>	[38]
75	COL6A2	MA	Glycine substitution	c.802G>T	p.G268C	Moderate Progressive UCMD	SSCD	<i>de novo</i>	Novel
76	COL6A2	MA	Glycine substitution	c.812G>A	p.G271D	Mild UCMD	SSCD	<i>de novo</i>	[3]
77	COL6A2	MA	Glycine substitution	c.820G>A	p.G274S	Mild UCMD	SSCD	Dominant	Novel
78	COL6A2	MA	Glycine substitution	c.821G>A	p.G274D	Mild UCMD	SSCD	<i>de novo</i>	Novel
79	COL6A2	MA	Glycine substitution	c.838G>C	p.G280R	Mild UCMD	SSCD	<i>de novo</i>	Novel
80	COL6A2	MA	Glycine substitution	c.839G>A	p.G280D	BM	SSCD	<i>de novo</i>	Novel
81	COL6A2	MA	Splicing	c.855+1G>A	p.G268_Q285del	Moderate Progressive UCMD	SSCD	<i>de novo</i>	[25]
82	COL6A2	MA	Splicing	c.856-2A>G	p.G286_K309del	Moderate Progressive UCMD	SSCD	Dominant	[3]
83-1 ^j	COL6A2	MA	Splicing	c.856-2A>G	p.G286_K309del	BM	SSCD	Dominant	[3]
83-2 ^j	COL6A2	MA	Splicing	c.856-2A>G	p.G286_K309del	BM	NA	Dominant	[3]
84	COL6A2	MA	Glycine substitution	c.866G>A	p.G289D	BM	SSCD	Dominant	[39]
85	COL6A2	MA	Glycine substitution	c.875G>T	p.G292V	Moderate Progressive UCMD	SSCD	<i>de novo</i>	[3]

Family	Gene	Mono or Bi-allelic	Category	Nucleotide change	Protein change	phenotype	COL6 IHC	Inheritance	Report
86	COL6A2	MA	Glycine substitution	c.893G>A	p.G298E	Early severe UCMD	SSCD	<i>de novo</i>	Novel
87	COL6A2	MA	Large deletion	c.900+102_1000-43del	p.G301_K333del	Moderate Progressive UCMD	SSCD	<i>de novo</i>	Novel
88	COL6A2	MA	Glycine substitution	c.901G>T	p.G301C	Moderate Progressive UCMD	SSCD	<i>de novo</i>	[3]
89	COL6A2	MA	Glycine substitution	c.902G>T	p.G301V	Mild UCMD	SSCD	<i>de novo</i>	Novel
90	COL6A2	MA	Glycine substitution	c.902G>A	p.G301D	Moderate Progressive UCMD	SSCD	<i>de novo</i>	[3]
91	COL6A2	MA	Glycine substitution	c.902G>A	p.G301D	Moderate Progressive UCMD	SSCD	<i>de novo</i>	[3]
92	COL6A2	MA	Glycine substitution	c.911G>T	p.G304V	BM	SSCD	<i>de novo</i>	Novel
93	COL6A2	MA	Missense	c.943G>A	p.D315N	Moderate Progressive UCMD	SSCD	<i>de novo</i>	Novel
94	COL6A2	MA	Missense	c.943G>A	p.D315N	BM	Normal	Dominant	Novel
95	COL6A2	MA	Splicing	c.950_954+8del	p.G310_K318del	Moderate Progressive UCMD	SSCD	<i>de novo</i>	Novel
96	COL6A2	MA	Splicing	c.955-2A>C	p.G319_K333del	Moderate Progressive UCMD	NA	<i>de novo</i>	Novel
97	COL6A2	MA	Splicing	c.955-2A>G	p.G319_K333del	Early severe UCMD	SSCD	<i>de novo</i>	[9]
98	COL6A2	MA	Splicing	c.955-2A>G	p.G319_K333del	Mild UCMD	SSCD	<i>de novo</i>	[9]
99-1 ^k	COL6A2	MA	Splicing	c.1053+1G>A	p.G334_R351del	BM	SSCD	Dominant	Novel
99-2 ^k	COL6A2	MA	Splicing	c.1053+1G>A	p.G334_R351del	BM	NA	Dominant	Novel
100	COL6A2	MA	Glycine substitution	c.1664G>A	p.G555E	Moderate Progressive UCMD	SSCD	<i>de novo</i>	Novel
101	COL6A2	MA	Small deletion	c.1858_1860del	p.I620del	Moderate Progressive UCMD	SSCD	<i>de novo</i>	Novel
102	COL6A2	MA	Missense	c.1861G>A	p.D621N	BM	SSCD	Dominant	[31]
103	COL6A2	MA	Missense	c.1870G>A	p.E624K	BM	Normal	<i>de novo</i>	[37]
104	COL6A2	MA	Missense	c.2192C>G	p.T731R	BM	SSCD	<i>de novo</i>	Novel
105	COL6A2	MA	Glycine substitution	c.2197G>A	p.G733R	BM	SSCD	<i>de novo</i>	[40]
106	COL6A2	MA	Missense	c.2271C>G	p.I757M	BM	Normal	<i>de novo</i>	Novel
107	COL6A2	MA	Small deletion	c.2741_2743del	p.F914del	BM	SSCD	<i>de novo</i>	Novel
108	COL6A2	MA	Missense	c.2978G>A	p.R993H	BM	SSCD	<i>de novo</i>	Novel
109	COL6A3	BA	Small deletion Small deletion	c.5692delG c.8737delG	p.V1898fs p.A2913fs	Early severe UCMD	CD	Recessive	[3]
110	COL6A3	MA	Missense	c.5525G>A	p.G1842E	BM	SSCD	Dominant	[7]
111	COL6A3	MA	Missense	c.5525G>A	p.G1842E	BM	SSCD	Dominant	[7]
112-1 ^l	COL6A3	MA	Missense	c.5525G>A	p.G1842E	BM	NA	Dominant	[7]
112-2 ^l	COL6A3	MA	Missense	c.5525G>A	p.G1842E	BM	NA	Dominant	[7]
113	COL6A3	MA	Missense	c.5525G>A	p.G1842E	BM	Normal	Dominant	[7]
114	COL6A3	MA	Splicing	c.6157-2A>G	p.G2053_P2070del	Early severe UCMD	SSCD	<i>de novo</i>	[3]
115	COL6A3	MA	Splicing	c.6157-2A>G	p.G2053_P2070del	Mild UCMD	SSCD	<i>de novo</i>	[3]
116	COL6A3	MA	Glycine substitution	c.6158G>T	p.G2053V	Moderate Progressive UCMD	SSCD	<i>de novo</i>	[10]
117-	COL6A3	MA	Missense	c.6199G>A	p.E2067K	BM	SSCD	Dominant	[21]

1 ^m										
117-2 ^m	COL6A3	MA	Missense	c.6199G>A	p.E2067K	BM	Normal	Dominant	[21]	
118	COL6A3	MA	Splicing	c.6210+1G>A	p.G2053_P2070del	Moderate Progressive UCMD	SSCD	de novo	[19]	

Table 1. (continued)

Family	Gene	Mono or Bi-allelic	Category	Nucleotide change	Protein change	Phenotype	COL6 IHC	Inheritance	Report
119	COL6A3	MA	Splicing	c.6210+1G>A	p.G2053_P2070del	Moderate Progressive UCMD	SSCD	<i>de novo</i>	[19]
120	COL6A3	MA	Splicing	c.6210+1G>A	p.G2053_P2070del	Early severe UCMD	SSCD	<i>de novo</i>	[19]
121	COL6A3	MA	Splicing	c.6210+1G>A	p.G2053_P2070del	Moderate Progressive UCMD	SSCD	<i>de novo</i>	[19]
122	COL6A3	MA	Splicing	c.6210+1G>A	p.G2053_P2070del	Mild UCMD	SSCD	<i>de novo</i>	[19]
123	COL6A3	MA	Splicing	c.6210+1G>A	p.G2053_P2070del	Moderate Progressive UCMD	SSCD	<i>de novo</i>	[19]
124	COL6A3	MA	Splicing	c.6210+2T>A	p.G2053_P2070del	Moderate Progressive UCMD	SSCD	<i>de novo</i>	[3]
125	COL6A3	MA	Glycine substitution	c.6212G>A	p.G2071D	Moderate Progressive UCMD	SSCD	<i>de novo</i>	[39]
126	COL6A3	MA	Glycine substitution	c.6247G>T	p.G2083C	Mild UCMD	SSCD	<i>de novo</i>	Novel
127	COL6A3	MA	Splicing	c.6309G>A	p.G2095_K2103del	Moderate Progressive UCMD	SSCD	<i>de novo</i>	Novel
128	COL6A3	MA	Splicing	c.6309+1G>A	p.G2095_K2103del	Moderate Progressive UCMD	SSCD	<i>de novo</i>	[25]
129	COL6A3	MA	Splicing	c.6310-2A>G	p.G2104_D2118del	Moderate Progressive UCMD	SSCD	<i>de novo</i>	Novel
130	COL6A3	MA	Splicing	c.6283-1G>T ⁿ c.6310-2A>T ⁿ	p.G2095_K2103delinsNSFLYLPVRLIPSL	Mild UCMD	SSCD	<i>de novo</i>	[27], [41]

IHC, immunohistochemistry; MA, mono-allelic; BA, bi-allelic; PTC, premature stop codon; NA, not available; UCMD, Ullrich congenital muscular dystrophy; BM, Bethlem myopathy; CD, complete deficiency; SSCD, sarcolemma-specific collagen VI deficiency; ^a brothers; ^b 26-2 and 26-3 are the sons of 26-1; ^d 40-2 is the father of 40-1; ^e 41-2 and 41-3 are the cousins of 41-1, and 41-4 and 41-5 are the sons of 41-2; ^f sisters; ^g 55-2 is the son of 55-1; ^h 56-2 is the daughter of 55-1; ⁱ brothers; ^j 83-2 is the mother of 83-1; ^k brothers; ^l brothers; ^m 117-2 is the mother of 117-1; ⁿ two variants on one allele.

Figures

Figure 1

Type and frequency of variants in collagen VI-related myopathy The proportion of (a) bi-allelic (BA) and mono-allelic (MA) variants, and (b) variants in COL6A1, COL6A2, and COL6A3. The frequencies of various types of (c) mono-allelic and (d) bi-allelic variants.

Figure 2

Schematic domain structure of collagen VI polypeptide chains and localization of the identified mono-allelic variants The identified missense variants and small in-frame deletions are indicated by triangles. Large genomic deletions, exon deletions by splicing variants, and pseudoexon insertions are indicated by rectangles. Previously reported variants are shown in pink and novel ones in yellow. A single cysteine residue (C) in each triple helical domain (THD) is important for molecular assembly. Most mono-allelic variants are clustered in the N-terminal side of or around the cysteine residue in the THD. (Figure is modified from Lampe et al. [9]).

Figure 3

Schematic diagrams and electropherograms at breakpoints of large genomic deletions in COL6A1 and COL6A2. We found a deletion of 216 bp (COL6A1) in transcripts in Family #3 and #4, and a deletion of 144 bp (COL6A1) and 99 bp (COL6A2) in transcripts in Family #5 and #87, respectively. At the genomic level, Family #3 and #4 carried a deletion of 1.2 kb spanning from IVS4-7 to IVS8+490 in COL6A1 (a). The 5' breakpoint of the 2.1 kb deletion found in Family #5 was located at the sixth base of exon 8 of COL6A1 and its 3' breakpoint was at -43 of intron 10 (b). One of the COL6A2 alleles of Family #87 contained a 1.2 kb deletion extending from IVS7+102 to IVS10-43 (c). E: exon sequence. The numbering of genomic positions at the breakpoints are based on the sequence from the Gene Reference Consortium GRCh37/hg19.

Figure 4

Representative muscle pathology of patients with pathogenic variants in triple helical domains. Histology of muscle from a control individual (a, e), a member of Family #87 with UCMD and a large genomic deletion (b, f), a member of Family #72 with BM with a glycine substitution in the triple helical domain (c, g), and a member of Family #109 with UCMD with bi-allelic small deletions in COL6A3 (d, h). Hematoxylin and eosin, scale bar 20 μm. (a–d) Immunostaining for

collagen VI, scale bar 10 μm (e–h). Immunofluorescence staining for collagen VI (green), PDGFR α (red), and laminin $\alpha 2$ (blue) in muscles of patients showing complete collagen VI deficiency (i, Family #64; j, Family #67; k, Family #109; l, Family #61; m, Family #62). Scale bar, 10 μm .