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Title

Diagnosis and aetiology of congenital muscular dystrophy: we are halfway there

Running head

Diagnosis of congenital muscular dystrophy

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Abstract

Objectives: To evaluate the diagnostic outcomes in a large cohort of congenital muscular dystrophy (CMD) patients using traditional and Next Generation Sequencing (NGS) technologies.

Methods: 123 CMD patients were investigated using the traditional approaches of histology, immunohistochemical analysis of muscle biopsy and candidate gene sequencing. Undiagnosed patients available for further testing were investigated using NGS.

Results: Muscle biopsy and immunohistochemical analysis found deficiencies of laminin α2, α-dystroglycan or collagen VI in 50% of patients. Candidate gene sequencing and chromosomal microarray established a genetic diagnosis in 32% (39/123). Of 85 patients presenting in the last 20 years, 28 of 51 who lacked a confirmed genetic diagnosis (55%) consented to NGS studies, leading to confirmed diagnoses in a further 11 patients. Using the combination of approaches, a confirmed genetic diagnosis was achieved in 51% (43/85). The diagnoses within the cohort were heterogeneous. 45/59 probands with confirmed or probable diagnoses had variants in genes known to cause CMD (76%), and 11/59 (19%) had variants in genes associated with congenital myopathies, reflecting overlapping features of these conditions. One patient had a congenital myasthenic syndrome and two had microdeletions. Within the cohort, five patients had variants in novel (*PIGY* and *GMPPB*) or recently published genes (*GFPT1* and *MICU1*) and seven had variants in *TTN* or *RYR1*; large genes that are technically difficult to Sanger sequence.

Interpretation: These data support NGS as a first-line tool for genetic evaluation of patients with a clinical phenotype suggestive of CMD, with muscle biopsy reserved as a second-tier investigation.

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Introduction

The congenital muscular dystrophies (CMDs) are inherited disorders of skeletal muscle characterized by hypotonia and weakness within the first 2 years of life, delayed gross motor milestones, and dystrophic features on skeletal muscle biopsy[1,2]. These disorders are phenotypically diverse and genetically heterogeneous. The boundaries between CMD, congenital myopathies and limb girdle muscular dystrophies are blurred, with overlap in disease genes, clinical presentation and histopathological features.

In 2008, Peat and colleagues published the protein and molecular diagnostic workup of an Australasian CMD cohort[2]. The diagnostic methods, muscle immunohistochemistry and Sanger sequencing of candidate genes, were described as "state-of-the-art" in an accompanying editorial[3]. Using this approach, an immunohistochemical classification was achieved in 45% (45/101) of the cohort and a genetic diagnosis in 24% (24/101). The advent of 'next generation sequencing' (NGS) has contributed to a rapid increase in the number of known CMD genes from 12 in 2008, to 28 in 2015[4]. However, the diagnostic yields of NGS by panel testing of known disease genes, whole exome, or whole genome approach remain uncertain.

This study evaluates the diagnostic outcomes in 123 CMD patients ascertained over 35 years. Of the 85 patients presenting in the last 20 years, 51 (60%) remained undiagnosed after conventional investigations. 28/51 (55%) were contactable and consented to NGS studies leading to confirmed genetic diagnoses in a further 11 of these 28 patients.

Methods

Patient Ascertainment

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CMD patients were identified retrospectively and prospectively through clinical records and the Institute for Neuroscience and Muscle Research (INMR) Biospecimen Bank. Clinical examination, review of medical records, muscle histology and complementary investigations such as blood creatine kinase level, brain MRI, muscle MRI and ultrasound imaging were used to define the clinical phenotype of affected individuals.

Inclusion Criteria: Evidence of muscle weakness and hypotonia within the first 2 years of life and clinical features consistent with congenital muscular dystrophy, such as delayed gross motor milestones, congenital/early contractures or scoliosis, brain MRI consistent with laminin-α2 deficiency or α-dystroglycanopathy, or a raised CK (>200 IU/L). Only the proband was included when more than one sibling was affected. Patients with a muscle biopsy performed between 1979 and 2014 were included if histopathological examination showed dystrophic changes, or non-specific myopathic findings, provided the clinical criteria were met. Six patients who fulfilled clinical criteria but were not investigated with a muscle biopsy were also included. Deliberately broad inclusion criteria were chosen to reflect the variable pathology which can occur early in the course of disease, secondary to selective muscle involvement and in specific subtypes such as collagen VI myopathies[5–6].

Inclusion in NGS studies was based on phenotype with preference given to families with multiple affected siblings and those with DNA available from the proband and parents. Inclusion of retrospective members of the cohort was limited by the need for additional consent and the availability of DNA samples. Given the elapsed time, it was considered insensitive to re-contact some families and some were no longer contactable, or the patient was deceased.

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Exclusion criteria: Structural changes in skeletal muscle diagnostic of a congenital myopathy, for example rods or cores. Eleven fetuses and neonatal deaths ascertained in the Peat cohort[2] were excluded because they had not been investigated further and we elected not to contact the families.

Immunohistochemical analysis

Immunohistochemical staining of the muscle biopsy for laminin-α2, glycosylated α-dystroglycan and collagen VI, was performed using previously reported methods[2]. Probands were classified as α-dystroglycanopathy, collagen VI-related myopathy or laminin-α deficient if their biopsy showed moderate to severely reduced, or absent staining.

Candidate gene sequencing

Candidate gene sequencing was performed on the basis of immunohistochemical classification, and when this was negative, was requested by the treating clinician on the basis of the clinical phenotype. Methods have been previously reported for *FKRP*[2], *LARGE*, *POMT1*, *POMT2*, *FKTN* and *POMGNT1*[7], the three collagen VI genes[8], *LAMA2*[9], *SEPN1*[10], *LMNA*[11], *DNM2*[12] and *ACTA1*[13].

Next generation sequencing (NGS)

NGS approach used was based on the evolving availability of different NGS approaches over the course of the study. Targeted NGS was performed with either a research-based 45 gene panel (Panel A) (supplemental methods), or a commercial 336 gene panel (Panel B) offered by PathWest Laboratory, Australia (Table e-5). WES was performed by the Broad Institute using previously published methods[11]. Whole genome sequencing (WGS) was performed on 3 probands who did not have a diagnosis following WES.

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Whole exome sequencing analysis pipeline.

Variant filtering was performed using the xBrowse web browser (https://atgu.mgh.harvard.edu/xbrowse). Variants were identified as outlined in Figure 1. A custom search was performed for all variants in the approximately 400 known neuromuscular disease genes at the time of investigation. When no candidate gene was identified, the whole exome was searched by inheritance pattern.

Candidate pathogenic variants were confirmed by Sanger sequencing in the proband and family members. The genetic diagnoses were reviewed by a cross-disciplinary team and were considered "confirmed" only when segregation was established in the family and the clinical phenotype, histology and immunohistochemical findings were consistent with the genetic change. The pathogenicity of the variant was determined based on *in silico* prediction programs, population frequency and reporting in locus-specific databases. In the case of novel variants and novel genes, protein specific experimental functional studies were performed in a tissue or animal model (Fig 1).

Standard Protocol Approvals and Patient Consents

Ethics approval for all aspects of this study was obtained from the Human Research Ethics Committee of the Sydney Children's Hospitals Network (Approval No: 10.CHW.45). Written informed consent was obtained from all participants and inclusion in NGS studies was dependent on completion of an additional specific consent, reflecting the complexities of NGS analysis.

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Results

Traditional approaches of immunostaining, Western blot and candidate gene sequencing provided a genetic diagnosis in 34%.

A cohort of 123 CMD patients was ascertained; 90 were part of the 1979-2006 cohort published by Peat and colleagues[2], and a further 33 probands were ascertained between 2006 and 2014 (Fig 2); 61 probands were female and 62 were male; 101 came from non-consanguineous families, 14 from consanguineous families and for 8 probands this information was not available. Eight probands had affected siblings.

Muscle histology was available for 117/123 probands (95%). The median duration from onset of symptoms to muscle biopsy was 18 months. Immunohistochemical analysis was performed on 113 muscle biopsy specimens; 57 probands (50%) could be classified to a CMD subtype based on a moderate or severe reduction in collagen VI, laminin-α2 or α-dystroglycan (Fig 3). Candidate gene sequencing was performed on the basis of clinical phenotype and immunohistochemical classification in 90 probands (Fig 3). The mean number of genes sequenced was 4 (range 1-16).

Using muscle biopsy and histological examination, immunohistochemical analysis, chromosomal microarray and candidate gene sequencing a genetic diagnosis was confirmed for 39 of 123 probands (32%) (Fig 2 and Fig 3). Two further patients had possible diagnoses: clinical and immunohistochemical findings were suggestive of a diagnostic subtype (Patient 33 with abnormal αDG and Patient 71 with abnormal laminin-α2), however, in both cases, only one heterozygous variant was identified on sequencing of candidate genes, and no further analysis had been performed.

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Next Generation Sequencing facilitates genetic diagnosis in CMD patients unsolved using traditional diagnostic approaches

Eighty two probands in the cohort remained undiagnosed after conventional investigation (see Fig 2). Because the cohort spans >35 years, including patients with onset of symptoms as early as 1969, many members of the cohort were lost to follow up, or deceased. When we restricted our analyses to patients presenting within the last 20 years (since our laboratory was established, n=85), follow up was more complete. 51 patients were undiagnosed by conventional investigation, of whom 28 were available for NGS studies (55%). Six were known to be deceased, and were not approached for consent, 14 were not contactable and three declined to participate.

Of the presenting after 1993, who consented to NGS testing (n=28), seven patients were investigated with a NGS neuromuscular gene panel (Panel A – 2, Panel B – 4, both panels - 1) and 21 with WES. DNA from the parents was included in WES studies in 18 cases and in three cases affected or unaffected siblings were included. Genetic diagnoses were established in 13/28 patients (see Fig 2 and Fig 3); 11 had confirmed diagnoses in known genes (3/11 by NGS panel and 8/11 by WES) and 2 had probable diagnoses of *TTN*-related myopathy with one Ambry class 4 and one class 3 variant. Two further patients had likely pathogenic variants in novel candidate genes (Table e-1). WGS has been performed in three probands undiagnosed following WES, but to date has not yielded a genetic diagnosis for these patients.

The subgroup of 28 patients investigated with NGS had remained undiagnosed despite extensive research-based candidate gene sequencing and was thus enriched for gene

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discovery. Two probands had variants in *GMPPB,* contributing to identification of this gene as a cause of α-dystroglycanopathy[14,15]. Patient 44, and her affected sister, had a homozygous recessive variant in *PIGY*, defining a novel multisystem disease secondary to a deficiency of GPI anchor biosynthesis[16]. A homozygous recessive variant in *ACTA1* was identified as a cause of congenital muscular dystrophy with rigid spine, a new phenotype of *ACTA1*-related disease[13]. Two patients had causative variants in genes published after enrolment in this study (*GFPT1* and *MICU1*)[17,18].

NGS facilitated diagnosis of *RYR1* or *TTN*-related disease in 5 patients, however the pathogenicity of the second variant found in 3 probands is uncertain. Both genes are large and have previously been technically difficult to Sanger sequence. Interpretation of pathogenicity of variants in these large genes remains challenging and is the subject of current research.

In Patient 98 a heterozygous missense variant in *LARGE* was identified by WES. His phenotype was consistent with α-dystroglycanopathy. CMA detected an intragenic deletion of *LARGE* (22q12.3(33,774,511-34,221,251)) inherited in trans, highlighting the complementary nature of these investigations.

Genetic diagnoses within the cohort are heterogeneous

In the total cohort a genetic diagnosis was established in 59/123 probands. This was confirmed in 54 probands and probable in 5 probands. 41 were made by traditional investigation and 18 by NGS techniques (Fig 2). The median age at diagnosis was 10 years (range 18 months – 42 years). The inheritance was *de novo* dominant in 24 probands and recessive in 35 probands.

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45 of the 59 probands (76%) with confirmed or probable genetic diagnoses had variants in a recognized CMD gene (Fig 3, Table e-2 and e-3). In 40/45 the gene was well known prior to this study (*FKRP*, *FKTN*, *LARGE* (2), *POMGNT1*, *POMT1* (2), *POMT2* (2), *LAMA2 (8)*; COL6 (15), *LMNA* (6), and *SEPN1* (2)). In the remaining five patients, the gene was identified as causing CMD during, or as a result of this study (*GMPPB* (2)*, MICU1*[18], *ACTA1*[13] and *PIGY*[16]).

Eleven probands (19%) had variants in genes previously known to be associated with congenital myopathies rather than CMD (*DNM2* (2), *RYR1* (4), *SIL1* (1), *ACTA1* (1), and *TTN* (3)). Patient 60 had variants in *GFPT1*, a recently published cause of a limb girdle myasthenia[17] (Table e-4).

Biochemical and pathological features predict likelihood of a genetic diagnosis

We assessed whether creatine kinase (CK) and dystrophic features on muscle biopsy are valuable diagnostic markers for CMD to assist in prioritization of patients for NGS and interpretation of results. CK measurements were available for 113/123 probands. Forty probands had CK levels >1000 IU/L on at least one occasion, 23 had mild elevation (200-1000 IU/L) and 50 had normal levels. A confirmed or probable genetic diagnosis was more likely in the 63 probands with a CK >200 IU/L (41/63, 65·1%) compared with those with a normal CK (17/50, 34%) (p=0·0004). Of the 15 patients with collagen VI-related CMD, 12 had a CK measuring ~200-1000 IU/L, none had a level >1000 IU/L, and 3 had normal levels.

Histological data was available for 117/123 probands. In 80/117 patients (68%), the muscle biopsy was classified as dystrophic, in 34 it showed non-specific abnormalities, and in three

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it was reported as normal. A confirmed or probable genetic diagnosis was more likely in the 80 probands with a dystrophic muscle biopsy (47/80, 58·8%) than in patients with a non-dystrophic biopsy (8/37 (21·6%); p=0·0002).

Both factors are considered in Table 1. Elevated CK was more commonly associated with a causative gene traditionally associated with CMD. All patients with *LAMA2*-related CMD, α-dystroglycanopathies, *LMNA*-related CMD, 1/2 with *SEPN1*-related CMD and 11/15 with collagen VI myopathies were within this group. The group of patients with dystrophic biopsies but normal CK included patients with mutations in *TTN*, *RYR1*, and *DNM2.* These genes are known to be associated with normal or mildly elevated creatine kinase levels and histological findings that can mimic dystrophic findings, such as centralized nuclei and fibre size variation[19-21].

Immunohistochemical analysis accurately predicts CMD subtype

A classification could be made on the basis of immunohistochemical analysis for 57/113 probands (50.4%) (Fig 3). Causative variants were identified in *COL6A1*, *COL6A2* or *COL6A3* in 13/20 probands (65%) classified as having a collagen VI-related disorder on the basis of moderate or severe reduction in collagen staining on IHC. One proband (Patient 58) was diagnosed with a *COL6A1* variant, but was missed by IHC classification because the collagen VI reduction was mild, rather than moderate or severe. *LAMA2* variants were found in 7/11 (64%) probands classified as having laminin-α2 deficiency on the basis of their IHC results. Patient 71 had a heterozygous variant only identified by Sanger sequencing and did not have WES. A genetic diagnosis was confirmed for 16/26 probands classified as having an α-dystroglycanopathy. Ten (38%) had variants in genes known to cause α-dystroglycanopathy (*FKRP*-1; *FKTN*-1; *LARGE* -2; *POMGNT1*-1; *POMT1*-2, *POMT2*-2;

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GMPPB-1) and 6 (23%) had variants in other genes (*DNM2*-2; *GFPT*-1; *RYR1*-3). One patient with a *GMPPB* mutation had not been classified as having an α-dystroglycanopathy by IHC because of a mild, rather than moderate or severe reduction.

Of the 113 patients investigated by IHC and/or Western blot analysis, a genetic diagnosis was more likely in the group with an immunohistochemical classification (38/57; 66·7%) compared with the group who could not be classified (16/56; 28·6%) ($p<0·001$). Antibodies to laminin-α2 and collagen VI were the most sensitive and specific. Moderate or severe reduction in α-dystroglycan was less specific, reflecting the technical difficulties of working with antibodies to glycosylated α-dystroglycan[22].

Discussion

This study describes a cohort of 123 potential CMD patients referred to a specialist neuromuscular diagnostic service over 35 years. Patients were initially investigated traditionally using muscle histology, immunohistochemical staining and Western blot of muscle biopsies, followed by candidate gene sequencing. In this large cohort an immunohistochemical classification could be made in only 51% (57/113). This was followed by candidate gene sequencing, which confirmed a diagnosis in only 32% of the cohort (39/123). Because of the retrospective nature of the cohort, many of the older patients, presenting as early as 1969, were deceased or lost to follow up. Of the 85 patients presenting in the last 20 years, 51 did not have a confirmed genetic diagnosis using candidate gene sequencing. 28/51 (55%) consented to NGS studies leading to confirmed diagnoses in a further 11 patients. Using the combination of approaches 51% (43/85) achieved a confirmed genetic diagnosis.

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A recently published ANN guideline for the evaluation and diagnosis of CMD recommended candidate gene testing for specific CMD subtypes, and consideration of WES as this technology becomes more accessible and affordable[23]. Candidate gene sequencing is both expensive and time consuming, and in this cohort it yielded a diagnosis in less than 40% of patients. Furthermore, the diagnoses within this cohort were heterogeneous. The more common CMD subtypes (collagen VI, laminin α2, α-dystroglycanopathies, *SEPN1*- and *LMNA*-related muscular dystrophy) which should be easily recognizable to a specialist neuromuscular clinician, comprised only 34% (42/123) of our cohort. 76% of diagnoses were in genes recognized as causes of CMD, but 24% had alternative diagnoses including genes better known as causes of congenital myopathy and one patient had a potentially treatable limb girdle myasthenic syndrome. These findings reflect the considerable overlap between the clinical and histological features of CMD and congenital myopathies.

The cost of next generation sequencing is falling rapidly and is commercially available in many centres. Use of NGS as a first line investigation has now become common practice in Australian paediatric neuromuscular centres with local availability of a gene panel covering almost 400 neuromuscular genes, with a minimum coverage of 90% to 20x, and average coverage to 94x, for Aus$1100 (USD 837). It is the investigation of choice in very young patients who are often deemed unfit for an invasive muscle biopsy. Of note the median duration from onset of symptoms to muscle biopsy was 18 months and the median age at diagnosis was 10 years using standard diagnostic approaches, providing further support for a rapid and non invasive test that can be done at any age. The genetic heterogeneity of our cohort also argues for the use of large, rather than selective panels, and using this panel we would expect to have detected 57 of the 59 confirmed or probable genetic diagnoses in our

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cohort. Two patients with micro-deletions would not have been detected, reinforcing the importance of CMA in detecting large-scale deletions not detected by NGS technology.

WES is also increasing in availability. Given the size of our cohort and bias in selecting probands for investigation by a gene panel versus WES, this study cannot draw conclusions about the relative efficacy of these different approaches. A previously published neuromuscular cohort study found a higher diagnosis rate for a 41gene panel compared with clinician-requested single gene testing (46% vs 15-19%)[27]. Coverage of the panel approach, with targeted capture of neuromuscular disease genes and Sanger fill-in of low-coverage exons, was better than WES, with 11 to 18% of pathogenic variants potentially missed by WES[27]. Challenges with coverage apply to both NGS panels and WES, and are frequently gene specific, with GC rich, repeat regions, and exon 1 often poorly covered. *FKRP* is a good example, with up to 40% of the gene not well covered. Although a targeted panel approach requires ongoing updating of the panel as new genetic causes of neuromuscular disease are identified, the approach remains appealing because incidental findings and variants of uncertain significance are minimized.

Increasingly, the role of the muscle biopsy and its timing in the diagnostic workup algorithm is being questioned. In our cohort, a genetic diagnosis was more likely in patients with an elevated CK, or dystrophic biopsy findings, however, neither was sensitive or specific. The muscle biopsy is expensive, invasive, can be challenging in infants and children with severe weakness and impaired respiratory function, and poses a risk of a malignant hyperthermia reaction for some patients. Immunohistochemical and Western blot analysis of biopsy specimens is resource intensive and has limited availability outside the research setting.

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The diagnostic yield of prospective investigation of neuromuscular patients with NGS without prior muscle biopsy is uncertain, and will be the subject of further study. The results of this cohort suggest it may be approximately 50%, with 59 of 123 patients having a diagnosis detectable by a NGS neuromuscular panel plus CMA. The largest previously published neuromuscular cohorts (incorporating a broader range of neuromuscular disorders) found a definitive genetic diagnosis for 49% (21/43) and 83% (29/35) using a 579 and 236 gene panel respectively[24,25]. In less selective cohorts the diagnosis rates are lower; 25% for 2000 consecutive patients referred to Baylor Genetics for WES for Mendelian disease[26]. The muscle biopsy will not become obsolete, but may not be required for up to 50% of patients who have a diagnosis readily made by genetic testing. Muscle biopsy offers a second tier investigation in patients negative on initial genetic testing, to help guide further investigation; and may be required to confirm the pathogenicity of novel variants by demonstrating reduced protein levels, or for RNA sequencing or cDNA analysis to prove splice site disruption (Fig 4).

Despite the enormous advances seen in genetic diagnosis over the last 10 years, a proportion of patients with CMD remain without a genetic diagnosis following NGS. The challenge of neuromuscular genetic research now lies with these unsolved families. Some may bear variants in known genes, missed because of low coverage or the difficulties of mapping repetitive sets of exons and short tandem repeats, and others may have complex genetic abnormalities, such as structural rearrangements, that may only be revealed via whole genome sequencing. As the more common causes of neuromuscular disease are identified, interpretation of rare variants in novel genes will be dependent on the strength of international collaboration, open access variant databases, common nomenclature for variants, and data sharing.

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Our success rate in diagnosing CMD has doubled over the past 10 years. As NGS enters routine clinical practice, it is transforming the traditional approach to diagnosis. Timely diagnosis has many benefits including the end of what is often a long diagnostic odyssey and can help change the focus from diagnosis to management of the child's difficulties[28]. Health care and medical surveillance for complications can be individualized[11] and families are provided with information which can restore reproductive confidence and be used in prenatal diagnosis. The challenge for health care service providers is to streamline access to NGS panels for referring clinicians as a first-line diagnostic enquiry and upskill clinicians in the interpretation of these results.

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Author contributions

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GO, ML, EO, MD, NL, EH, DM, NC, SC, and KN contributed to conception and design of the study. GO, ML, SL, LW, LW, JP, RG, SS, HB, SK, MD, NL, FM, DM, NC, SC, KN, contributed to acquisition and analysis of data. GO, SC and KN contributed to drafting of the text and preparation of the figures.

Potential conflicts of interest

The authors report no conflicts of interest.

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Figure legends:

Figure 1: Whole exome sequencing analysis pipeline

Figure 2: Cohort ascertainment and investigation

123 CMD patients were ascertained. 101 were part of a cohort published by Peat et al*. 11 fetuses and deceased neonates were excluded. Conventional investigation was with protein-based screening of muscle biopsy specimens, candidate gene sequencing and chromosomal microarray (CMA). Undiagnosed patients were investigated with Next Generation Sequencing (NGS) technologies, when available for consent. Follow up was more complete in patients presenting with onset of disease after 1993.

* These patients were included in the cohort published by Peat et al, Diagnosis and etiology of congenital muscular dystrophy, Neurology. 2008; 71:312-321

Figure 3: Diagnostic outcomes

A. Immunohistochemical analysis, candidate gene sequencing and genetic diagnoses:

Panel A illustrates the classification of patients by immunohistochemical (IHC) analysis and diagnoses made by candidate gene sequencing and next generation sequencing (NGS).

Left panel (IHC classification): 113 probands had immunohistochemical (IHC) analysis performed on muscle biopsy specimens. 57 probands were able to be classified on the basis of a moderate or severe reduction in collagen VI, laminin-α2 or glycosylated α-dystroglycan. 56 patients could not be classified by IHC analysis. Middle panel (Candidate gene sequencing): Candidate gene sequencing was performed on the basis of IHC classification, and when unclassified, on the basis of clinical phenotype. The gene sequenced is indicated in a white box, and the confirmed genetic diagnoses are shown by grey boxes. The number of patients undiagnosed after candidate gene sequencing is shown in a grey circle.

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Right panel (NGS): NGS was performed on the number of patients indicated with a white circle. The confirmed diagnoses are shown in grey.

B. Heterogeneity of genetic diagnoses in a congenital muscular dystrophy cohort:
Of the patients who had a confirmed or probable genetic diagnosis, 76% of the cohort had variants in genes previously recognized, or recently described as causes of congenital muscular dystrophy. 19% of patients had variants in genes better recognized as causes of congenital myopathy. One patient had a congenital myasthenic syndrome with compound heterozygous variants in *GFPT1*. Two patients had de novo microdeletions.

* Includes one patient with probable *LARGE*-CMD, but with only a heterozygous variant detected in *LARGE*.

+ Includes one patient with probable *LAMA2*-CMD, but with only a heterozygous variant in *LAMA2*.

[x] Includes two patients with probable *TTN* myopathy, with a second variant of uncertain pathogenicity/Ambry Class 3 variant.

Figure 4: Proposed diagnostic algorithm for Congenital Muscular Dystrophy

Proposed diagnosis of suspected congenital muscular dystrophy patients using a targeted next generation sequencing neuromuscular gene panel after exclusion of diagnoses missed by this technology. Muscle biopsy should be considered in patients undiagnosed by NGS.
CMA, chromosomal microarray; SMA, spinal muscular atrophy; NGS, next generation sequencing; RNA, RNA sequencing

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Table 1 Review of genetic diagnoses by muscle biopsy findings and creatine kinase levels.

	Elevated CK (>1000 IU/L)	Mild elevation (200-1000I U/L)	Normal CK (<200IU/L)
Dystrophic muscle biopsy			
Dystrophic (including variation in fibre size, increased internalized nuclei, degenerating and regenerating fibres and increased interstitial connective tissue)	*LAMA2* (6) *FKRP* (1) *FKTN* (1) *POMT1* (1) *POMT2* (2) *POMGNT1* (1) *LARGE* (2) *GMPPB* (2) *LMNA* (1) *ACTA1* (1) Undiagnosed (13)	*COL6A1* (6) *COL6A2* (3) *COL6A3* (2) *LMNA* (3) *SEPN1* (1) *PIGY* (1) Undiagnosed (2)	*COL6A2* (1) *COL6A3* (1) *LAMA2* (1) *DNM2* (1) *GFPT1* (1) *RYR1* (2) Candidate (1) Undiagnosed (8)
Dystrophic – endstage with extensive fibro-fatty replacement of tissue	Undiagnosed (1)	Undiagnosed (1)	*TTN* (1) Undiagnosed (1)
Dystrophic + autophagocytic vacuoles			Undiagnosed (1)
Dystrophic + focal cytoplasmic vacuoles and increased glycogen accumulation	*POMT1* (1)		
Dystrophic + intranuclear rods on EM (missed on initial review)			*ACTA1* (1)
Dystrophic + minicores			Undiagnosed (1)
Dystrophic + prominent central nuclei			*TTN* (2)
Dystrophic + Z-band streaming		Undiagnosed (1)	Undiagnosed (1)
Non-dystrophic			
Increased variation in fibre size		*SIL1* (1) Undiagnosed (1)	*COL6A2* (1) Candidate (1) Undiagnosed (7)
Increased variation in fibre size, incl abnormally large muscle fibres	Undiagnosed (1)		Candidate (1) Undiagnosed (3)
Increased variation in fibre size, increased connective tissue			*RYR1* (1) Undiagnosed (1)
Increased variation in fibre size, increased central nuclei	Undiagnosed (1)		*DNM2* (1) Undiagnosed (1)
Increased variation in fibre size, type 1 fibre predominance			Microdeletion (1) Undiagnosed (1)
Type 1 fibre predominance	*MICU1* (1)		*SEPN1* (1) Undiagnosed (3)
Type 1 fibre predominance, increased central nuclei			Undiagnosed (1)
Normal			Microdeletion (1)

Excluded are 16 patients who either did not have a muscle biopsy, or for whom, the CK result had not been recorded.

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Supplementary Tables

Table e-1 Clinical and histological characteristics and genetic diagnoses of the cohort

ID	Sex	Age (years)	Mode of Dx	Clinical features	CK[z]	Histology	IHC	Gene	Mode	Mutation	Inheritance
1*	F	41	CGS	Onset age 8mo, gross motor delay, deterioration with loss of ambulation, severe scoliosis	Normal	Non-specific	U	*SEPN1* NM_020451.2	AR	Hom. c.943G>A, p.(Gly315Ser) LOVD ID #12362[a]	Parents heterozygous Affected sister hom.
2*	F	38		Onset age 2y, gross motor delay, severe scoliosis	Normal	Non specific	U				
3*	F	1y 6mo[+]		Onset age 6mo, sat, but never walked, global delay	U	Non-specific	αDG				
4*	F	12[+]		Onset age 1y, gross motor delay, scoliosis	Normal	Dystrophic	U				
5*	M	32	WES	Infantile hypotonia and gross motor delay, mild facial weakness, mild ptosis, contractures, scoliosis, biventricular impairment, restrictive lung disease	U	Dystrophic	αDG	*RYR1*[U] NM_000540.2	AR	g.38933095T>C, c.270+2T>C (RNA seq studies pending); c.6721C>T, p.(Arg2241X) LOVD ID #54662	Parents heterozygous
6*	M	31		Infantile hypotonia, gross motor delay, mild facial weakness	Normal	Dystrophic	U				
7*	M	32		Infantile hypotonia, gross motor delay, walked age 3y, ophthalmoplegia	Elevated 789-1057	Dystrophic	COLVI				
8*	M	29		Infantile hypotonia, congenital hip dysplasia and femur fracture, gross motor delay, walked age 3y, facial weakness, contractures, gastrostomy fed, mild cognitive delay	U	Non-specific	U				
9*	M	31		Onset age 18mo, gross motor delay, contractures, seizures	Elevated 718-3044	Dystrophic	U				
10*	F	1y 7mo[+]		Infantile hypotonia, arthrogryposis, gross motor delay, facial weakness, contractures, restrictive lung disease	Normal	Dystrophic	αDG				
11*	F	10[+]		Infantile hypotonia, gross motor delay, stood with support age 3.5y, profound cognitive delay	Normal	Non-specific	U				
12*	M	28	CGS	Onset age 18mo, gross motor delay, mild cognitive delay	Elevated 1453-1894	Dystrophic	αDG	*POMT2* NM_013382.5	AR	c.2243G>C, p.(Trp748Ser); c.551C>T, p.(Thr184Met) LOVD ID #10032	Parents heterozygous
13*	F	28		Infantile hypotonia, gross motor delay, sat age 3y, contractures, scoliosis, gastrostomy fed, profound cognitive delay	Normal	Dystrophic	U				
14*	F	27		Infantile hypotonia, gross motor delay, walked age 6y, mild facial weakness	Normal	Non-specific	U				
15*	F	29		Onset age 6mo, gross motor delay, moderate developmental delay	Elevated 2900	Dystrophic	αDG				
16*	M	25	CGS	Onset age 6mo, gross motor delay, progression with wheelchair dependency in teenage years, mild facial weakness	Elevated 863-1710	Dystrophic	U	*LMNA* NM_170707.3	AD	c.1366A>G, p.(Asn456Asp) LOVD ID #54663	De novo
17*	F	24		Infantile hypotonia, gross motor delay, standing with support at 4y, facial weakness, contractures,	Elevated 63-7121	Dystrophic	Laminin-α2				
18*	M	23		Infantile hypotonia and weakness, bilateral congenital humeral fractures	Mild elevation 200-400	Dystrophic	COLVI				
19*	F	23		Weakness, severe scoliosis, seizures	U	Normal	U				
20*	F	24	Panel B	Onset age 6mo, gross motor delay, walked age 3y, mild facial weakness, choreoathetosis, rhabdomyolysis, moderate developmental delay	Elevated 943-6728	Dystrophic	U	*GMPPB* NM_013334.3	AR	c.79G>C, (p.Asp27His); c.1036C>A, (p.Arg346Ser) LOVD ID #54664	Parents heterozygous

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1*	F	24		Infantile hypotonia, gross motor delay, walked age 2y, contractures, distal laxity, scoliosis, eosinophilic oesophagitis	Elevated 1405	Dystrophic	αDG				
2*	M	2y 6mo[+]		Infantile hypotonia, gross motor delay, facial weakness, contractures, chronic respiratory failure	Elevated 569-1143	Dystrophic	U				
3*	M	23	CGS	Infantile hypotonia, congenital femur fracture, walked age 2 years, progression with wheelchair dependency by 12y, distal laxity, hyperkeratosis pilaris, restrictive lung disease	Mild elevation 282	Dystrophic	COLVI	*COL6A3* NM_004369.3	AD	g.238269763C>T, c.6210+1 G>A LOVD ID #54694	De novo
4*	F	23		Infantile hypotonia, wheelchair dependency, contractures, severe developmental delay, polymicrogyria	Elevated 2756-3371	Dystrophic	Laminin-α2				
5*	M	25		Generalized weakness, facial weakness, congenital heart disease, moderate cognitive delay	Normal	Non-specific	U				
6*	F	21	Panel A	Onset age 6mo, never walked, contractures, distal laxity	Normal	Non-specific	COLVI	*COL6A2* NM_001849.3	AD	c.954G>T, p.(Lys318Asn) LOVD ID #54695	De novo
7*	F	21		Infantile hypotonia, gross motor delay, walked with support at 3 years, facial weakness, distal laxity, scoliosis, gastrostomy, moderate cognitive delay, seizures, MRI - T2 white matter hyperintensities	Elevated >2000	Dystrophic	Laminin-α2				
8*	F	22	CGS	Onset age 6mo, gross motor delay, mild facial weakness, scoliosis, cognitive delay, MRI - T2 white matter hyperintensities	Elevated 2500	Dystrophic	Laminin-α2	*LAMA2* NM_000426.2	AR	Report not available	
9*	F	20		Onset age 7mo, gross motor delay, gastrostomy fed, moderate cognitive delay	Normal	Non-specific	U				
0*	M	22		Infantile hypotonia and weakness, arthrogryposis, congenital radial fracture, congenital hip dislocation, contractures, scoliosis, severe restrictive lung disease	U	Non-specific	U				
1*	M	21	WES	Onset age 2y, gross motor delay, mild facial weakness, dense bilateral cataracts, mild cognitive delay, seizures	Elevated 4344-5200	Dystrophic	αDG	*GMPPB* NM_013334.2	AR	Hom. c.553C>T, p.(Arg185Cys) LOVD ID #30775[b]	Parents heterozygous
2*	M	36	Panel A	Onset age 2y, gross motor delay, slowly progressive weakness, contractures, scoliosis, spinal rigidity	Elevated 396-1930	Dystrophic	U	*ACTA1* NM_001100.3	AR	Hom. c.460G>C, p.(Val154Leu) LOVD ID #31035[c]	Parents heterozygous
3*	F	19	CGS	Onset 1y, gross motor delay, walked age 6y with support, moderate cognitive delay, leukodystrophy	Elevated 5000-7000	Dystrophic	αDG	*LARGE*[U] NM_004737.4	AR	c1640G>A, p.(Arg547His) + ? LOVD ID #54665	
4*	M	19		Infantile hypotonia, gross motor delay, contractures, intellectual disability, seizures	Normal	Non-specific	U				
5*	M	24	WES	Infantile hypotonia, gross motor delay, walked with support at 6y, extreme hypermobility and distal laxity, joint dislocations, scoliosis	Normal	Non-specific	U	Candidate	AR		Parents heterozygous
6*	M	20	CGS	Onset age 6mo, gross motor delay, walked with support at 4y, ataxia, congenital cataracts, cerebellar hypoplasia and atrophy	Mild elevation 215-309	Non-specific	U	*SIL1* NM_001037633.1	AR	c.178G>T, p.(Glu60X); c.346delG (p.Gly116AlafsX42) LOVD ID #54666	Parents heterozygous
7*	F	19		Onset age 6mo, gross motor delay, contractures	Elevated 618-1040	Dystrophic	U				
8*	F	19	CGS	Onset age 6mo, gross motor delay, walked at 2.5y, contractures, moderate cognitive delay, seizures, MRI - Walker Warburg syndrome	Elevated 996-2824	Dystrophic	αDG	*POMGNT1* NM_001243766.1	AR	c.1100G>A, p.(Arg367His); g.46657769C>T, c.1539+1G>A LOVD ID #9927	Parents heterozygous
9*	M	20		Infantile hypotonia, gross motor delay, arthrogryposis, contractures, scoliosis, gastrostomy fed, profound cognitive delay, visual impairment, seizures, MRI - progressive white matter loss	U	Non-specific	U				
0*	M	20	WES	Infantile hypotonia, cryptorchidism, gross motor delay, walked age 2y, facial weakness, hypermobility and	Normal	Non-specific	U				

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				distal laxity, mild spinal rigidity, moderate cognitive delay							
1*	M	18	CGS	Onset age 6mo, congenital hip dislocation, gross motor delay, walked just prior to 6mo, mild facial weakness	Normal	Dystrophic	αDG	*RYR1* NM_000540.2	AR	c.6443C>T, p.(Ser2148Phe); c. 7361G>A, p.(Arg2454His) LOVD ID #54667	Parents heterozygous
2*	F	3+		Infantile hypotonia, gross motor delay, never stood, mild facial weakness, contractures, laryngeal cleft, profound cognitive delay	Normal	Dystrophic	U				
3*	F	45		Infantile hypotonia, gross motor delay, never sat, arthrogryposis, contractures	Normal	Non-specific	U				
4*	F	2+	WES	Infantile hypotonia, profound global delay, arthrogryposis, seizures	Mild elevation 323-554	Dystrophic	U	*PIGY* NM_001042616	AR	Hom. c.137T>C, p.(Leu46Pro) LOVD ID #54668[d]	Parents heterozygous
5*	F	17	CGS	Onset age 8mo, congenital hip dislocation, gross motor delay, contractures	Elevated 5735-9000	Dystrophic	αDG	*FKTN* NM_001079802.1	AR	c.340G>A, p.(Ala114Thr); c.859delA, p.(Thr286fs) LOVD ID #9918	Parents heterozygous
6*	M	29	CGS	Infantile hypotonia, gross motor delay, walked age 4y, non-ambulant from 11y, facial weakness, ophthalmoplegia, contractures, scoliosis	Normal	Non-specific	αDG	*RYR1* NM_000540.2	AR	c.6721C>T, p.(Arg2241X); g.38997345T>G, c.8693-124T>G, p.(Gly2898Aspfs) LOVD ID #54669	Parents heterozygous
7*	F	18	CGS	Onset age 16mo, progression with loss of ambulation at 10y, contractures, scoliosis, restrictive lung disease	Mild elevation 448	Dystrophic	U	*LMNA* NM_170707.3	AD	c.810G>T, p.(Lys270Asn) LOVD ID #54670	De novo
8*	M	16	Panel B	Infantile hypotonia, mild facial weakness, mild facial weakness, arthrogryposis, distal laxity and hypermobility	Normal	Dystrophic	U				
9*	M	22	CGS	Onset age 6mo, progressive course with loss of ambulation from 10y, mild facial weakness, scoliosis, restrictive lung disease	Mild elevation 57-503	Dystrophic	U	*SEPN1* NM_020451.2	AR	Hom. c.1397G>A, p.(Arg466Gln) LOVD ID #54671	Parents heterozygous
0*	F	4+		Infantile onset, arthrogryposis, contractures, scoliosis, profound cognitive delay, gastrostomy	Elevated 105-1535	Dystrophic	U				
1*	F	14	CGS	Onset age 3mo, gross motor delay, contractures, nocturnal ventilation, MRI - cobblestone lissencephaly	Elevated 487-3260	Dystrophic	Laminin-α2	*LAMA2* NM_000426.2	AR	c.4523G>C, p.(Arg1508Thr); c.4645C>T, p.(Arg1549X) LOVD ID #54672	Parents heterozygous
2*	M	16	WES	Infantile onset, facial weakness, ptosis, mild cognitive delay	Normal	Non-specific	U	Candidate	X-linked		Mother heterozygous
3*	M	15	Declined	Infantile hypotonia, proximal weakness, cognitive delay	Elevated 2500-5300	Non-specific	U				
4*	F	21		Gross motor delay, deterioration from 7y, mild facial weakness, arthrogryposis, contractures, short stature, spinal rigidity, ovarian failure, microcephaly	Mild elevation 400	Dystrophic	U				
5*	M	28		Infantile onset, weakness, mild facial weakness, distal laxity	Mild elevation 598-798	Non-specific	U				
6*	M	22	CGS	Infantile hypotonia, eventration of diaphragm, gross motor delay, mild facial weakness, ptosis, ophthalmoplegia, contractures, gastrostomy fed	Normal	Dystrophic	αDG	*DNM2* NM_001005360.2	AD	c.1880C>A, p.(Pro627His) LOVD ID #54673[e]	De novo
7*	F	15		Infantile onset, gross motor delay, distal laxity	Mild elevation 290	Dystrophic	COLVI				
8*	M	15	CGS	Onset age 18mo, weakness, hypermobility and distal laxity	Mild elevation 265-306	Dystrophic	U	*COL6A1* NM_001848.2		c.877G>A p.Gly293Arg LOVD ID #54696	De novo
9*	F	41	CGS	Gross motor delay, mild facial weakness, distal laxity, scoliosis,	Elevated 4100	Dystrophic	αDG	*FKRP* NM_024301.2	AR	c.826C>A, p.(Leu276Ile); c.162_165dupGGAG, p.(Phe56GlyfsX6) LOVD ID #22941	Parents heterozygous

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0*	F	13	WES	Congenital hypotonia, gross motor delay, mild facial weakness, contractures, scoliosis	Normal	Dystrophic	αDG	*GFPT1* NM_001244710.1	AR	g.69581446T>C, c.686-2A>G; c.1072A>G, p.(Met358Val) LOVD ID #54674	Parents heterozygous
1*	M	13	CGS	Gross motor delay and hypotonia, proximal weakness, MRI - delayed myelination and enlarged extra-axial spaces	Elevated 3275-7806	Dystrophic	αDG	*POMT1* NM_007171.3	AR	c.193G>A, p.(Gly65Arg); c.1847_1849delGGT, p.(Trp616del) LOVD ID #9968	Parents heterozygous
2*	F	14	CGS	Onset age 23mo, proximal weakness, distal laxity, round face, prominent heels and hyperkeratosis pilaris	Mild elevation 395	Dystrophic	COLVI	*COL6A1* NM_001848.2	AD	c.887G>T, p.(Gly296Val) LOVD ID #54697	De novo
3*	M	14	CGS	Onset age 2y, congenital hip dislocation, proximal weakness, distal laxity, contractures	Mild elevation 475	Dystrophic	COLVI	*COL6A3* NM_004369.3	AD	c.6284 G>T p.(Gly2095Val) LOVD ID #54698	De novo
4*	M	12		Infantile onset, weakness	Normal	Dystrophic	U				
5*	F	17		Proximal weakness, moderate developmental delay	Elevated 994-1618	Dystrophic	αDG				
6*	F	12	CGS	Infantile onset, gross motor delay, proximal weakness, facial weakness, ptosis, ophthalmoplegia, contractures	Normal	Non-specific	αDG	*DNM2* NM_001005360.2	AD	c.1102G>A, p.(Glu368Lys) LOVD ID #54675[e]	De novo
7*	F	34		Infantile onset, generalized weakness, deterioration from teenage years, contractures, scoliosis	U	Dystrophic	αDG				
8*	F	18	CGS	Infantile hypotonia, congenital hip dislocation, gross motor delay, mild facial weakness, distal laxity, scoliosis, hyperkeratosis pilaris, long finger flexor contractures	Normal	Dystrophic	COLVI	*COL6A2* NM_001849.3	AD	g.47533989T>C, c.801+2T>C LOVD ID #54699	De novo
9*	F	12	CGS	Infantile hypotonia, arthrogryposis, congenital femur fracture and hip dislocation, gross motor delay, walked age 2 years, mild facial weakness, contractures, distal laxity, hyperkeratosis pilaris, restrictive lung disease	Mild elevation 378	Dystrophic	COLVI	*COL6A2* NM_001848.2	AR	c.1855_1860delGTCATC, p.(Val619_Ile620del); g.47545179G>T, c.1771-1G>T LOVD ID #54700	Parents heterozygous
0*	M	12	CGS	Infantile onset, gross motor delay, distal laxity, contractures, hyperkeratosis pilaris, prominent heels	Mild elevation 245	Dystrophic	COLVI	*COL6A1* NM_001848.2	AR	Hom. c.1660delG, p.(Asp554ThrfsX48) LOVD ID#54701	Parents heterozygous
1*	M	18	CGS	Infantile onset, gross motor delay, walked at 4 years, contractures, spinal rigidity	Normal	Dystrophic	Laminin-α2	*LAMA2*[U] NM_000426.2	AR	c.4860G>A, p.(Phe1573Ser fs49X); + ? LOVD ID #54676	Paternally inherited
2*	F	1y 2mo[+]		Infantile hypotonia and weakness, contractures, respiratory weakness	Elevated 1092-2367	Non-specific	αDG				
3*	F	20[+]	WES	Onset age 1y of age with proximal weakness, progressive with loss of ambulation at 17 years, distal laxity replaced by progressive contractures, scoliosis, severe restrictive lung disease	Normal	Dystrophic	U				
4*	M	6mo[+]		Infantile hypotonia and weakness, contractures, ptosis, respiratory weakness	Normal	Dystrophic	U				
5*	M	11	Declined	Onset age 7mo, gross motor delay, never stood, mild facial weakness, MRI - white matter abnormalities	Elevated 3893	Dystrophic	Laminin-α2				
6*	M	1mo[+]		Infantile onset hypotonia and weakness, bulbar dysfunction and respiratory weakness	U	Normal	U				
7*	M	15	CMA	Infantile onset hypotonia, gross motor delay, walked at 24mo, distal laxity and hypermobility, contractures, extrapyramidal movements from 18mo	Normal	Non-specific	U	Microdeletion	AD	14q13.2-q21.1 (35,327,739-38,602,335)	De novo
8*	M	10		Infantile hypotonia, proximal weakness, mild facial weakness, gastrostomy	Normal	Dystrophic	U				
9*	M	11	CGS	Onset age 7mo, weakness, contractures, MRI - white matter T2 hyperintensities, cerebellar cysts	Elevated 6752	Dystrophic	αDG	*POMT2* NM_013382.5	AR	c.1997A>G, p.(Tyr666Cys); c.1238G>C, p.(Arg413Pro) LOVD POMT2_00013	Parents heterozygous
0*	F	2mo[+]		Infantile hypotonia and weakness, arthrogryposis, congenital hip dislocation, brain findings suggestive of CMD at autopsy	Normal	Dystrophic	U				

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1*	M	13	CMA	Infantile hypotonia, arthrogryposis, congenital hip dysplasia, gross motor delay, walked age 3y, contractures, hypermobility, scoliosis	Normal	Normal	U	Microdeletion	AD	6q13-q14.1 (75,540,881 - 83,110,141) incl *COL12A1*	De novo
2*	F	26		Infantile hypotonia, bilateral pes cavus, left hip dislocation, gross motor delay, walked with support at 18mo, scoliosis	Normal	Dystrophic	U				
3*	M	22	Declined	Infantile hypotonia, proximal weakness, contractures, mild scoliosis, visual impairment	Elevated 2283	Dystrophic	αDG				
4*	M	23	Panel A	Onset age 6mo, proximal weakness, mild scoliosis	Elevated 3195	Dystrophic	Laminin-α2	*LAMA2* NM_000426.3	AR	c.7691T>C, p.(Leu2564Pro); c.9253C>T, p.(Arg3085X) LOVD ID #54677	Parents heterozygous
5*	M	10	CGS	Infantile hypotonia, arthrogryposis, congenital hip dislocation, gross motor delay, walked with support at 3 years, mild facial weakness, ptosis, contractures, mild scoliosis	Normal	Dystrophic	COLVI	*COL6A3* NM_004369.3	AD	c.6221G>A, p.(Gly2074Asp) LOVD ID #54702	De novo
6*	M	10	WES	Infantile hypotonia, cryptorchidism, gross motor delay, hypermobility, hyperkeratosis pilaris	Normal	Non-specific	U				
7*	F	25	Panel B	Onset age 4mo, gross motor delay, contractures,	Elevated 1146	Dystrophic	NA				
8*	F	23		Infantile hypotonia and weakness, pulmonary artery stenosis	U	Non-specific	αDG				
9*	F	1mo^{+}		Infantile onset hypotonia and weakness, contractures, respiratory weakness	U	Non-specific	U				
0*	M	22	CGS	Onset age 6mo, generalized weakness, mild facial weakness, hypermobility and distal laxity, contractures, hyperkeratosis pilaris	Mild elevation 375-603	Dystrophic	COLVI	*COL6A2* NM_001849.3	AD	c.785G>A, p.(Gly262Asp) LOVD ID #54703	De novo
1	M	44	Panel A	Infantile hypotonia and weakness, mild facial weakness, ptosis, contractures, dilated cardiomyopathy in 20s	Normal	Dystrophic	U	*TTN* NM_001267550.1	AR	g.179499190_179499193delGAAA, c. 42315_42318del, p.(Lys14105Asnfs); g.179444577T>G, c. 67349-2A>C LOVD ID #54678	Presumed paternally inherited (deceased) Maternally inherited
2	F	21	CGS	Onset age 18mo, progressive weakness with loss of ambulation age 10y, facial weakness, ptosis, contractures, tachyarrhythmia requiring pacemaker, restrictive lung disease	Mild elevation 650-900	Dystrophic	U	*LMNA* NM_170707	AD	c.116A>G, p.(Asn39Ser) LOVD ID #54679	De novo
3	M	17	WES and WGS	Infantile hypotonia and weakness, arthrogryposis, congenital humeral and clavicle fracture, gross motor delay, walked age 6y, mild facial weakness, distal laxity, contractures, scoliosis, mild learning difficulties	Normal	Dystrophic	COLVI	*TTN*^{U} NM_001267550.1	AR	c.25335dup, p.(Lys8446GlnfsX8); c.45328G>A, p.(Asp15110Asn) and c.23177C>T, p.(Ser7726Leu) LOVD ID #54680	Paternally inherited; Maternally inherited.
4	F	13	WES	Infantile onset hypotonia, arthrogryposis, inguinal hernia, umbilical hernia, gross motor delay, walked age 3y, distal laxity, contractures, scoliosis	Normal	Non-specific	COLVI				
5	M	9	CGS	Infantile hypotonia and weakness, arthrogryposis, congenital hip dislocation, gross motor delay, contractures	Elevated 2600	Dystrophic	Laminin-α2	*LAMA2* NM_000426.2	AR	c.2556delT, p.(Phe852LeufsX36); c.6919_6920delTA, p.(Tyr2307LeufsX2) LOVD ID #54681	Parents heterozygous
6	M	11	Panel A Panel B	Onset age 6mo, gross motor delay, walked age 2y11mo, facial weakness, distal laxity, contractures, scoliosis, rigid spine, dilated cardiomyopathy, restrictive lung disease	Normal	Dystrophic	U	*TTN*^{U} NM_001267550.1	AR	g.179466399del, c.55418delG, p.(Arg18473fsX14); g.179433110_179433127del, c.77732_77749del, p.(Ser25911_Leu25916del) LOVD ID #54682	Paternally inherited; Maternally inherited
7	M	9	WES	Onset age 6mo, gross motor delay, distal laxity, hyperreflexia	Normal	Non-specific	αDG				

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M	9	WES + CMA	Infantile hypotonia, gross motor delay, MRI - white matter abnormalities	Elevated	Dystrophic	αDG	*LARGE* NM_004737.4	AR	c.1640G>A, p.(Arg547His); Intragenic deletion (22q12.3(33,774,511-34,221,251) LOVD ID #54683	Parents heterozygous
M	20	WES	Onset age 1y, gross motor delay, walked age 3y, progressive weakness, facial weakness, bulbar dysfunction, gastrostomy, aortic root dilatation, restrictive lung disease	Normal	Dystrophic	U	Candidate	AD		De novo
M	9	CGS	Infantile hypotonia and weakness, gross motor delay, walked age 20mo, mild facial weakness, distal laxity, contractures, hyperkeratosis pilaris, finger flexor contractures	Mild elevation 419	Dystrophic	COLVI	*COL6A1* NM_001848.2 *COL6A2* NM_001849.3	AD	g.47407557_47407577del, c.793_804+9del21, p.(Pro254_Glu268del15); g.47545696C>A, c.1970-3C>A, p.(Gly657_Arg698del42) LOVD ID #54705	De novo
F	14	CGS	Infantile hypotonia, congenital hip dislocation, talipes equinovarus, proximal weakness, hypermobility, distal laxity, mild scoliosis, hyperkeratosis pilaris	Mild elevation 343-405	Dystrophic	COLVI	*COL6A2* NM_001849.3	AR	c.316G>A, p.(Glu106Lys); c.736_801del66, p.(Cys246_Lys267del22) LOVD ID #54706	Paternally inherited De novo
F	8	WES	Onset age 18mo, walked age 22mo, contractures, hepatosplenomegaly, moderate intellectual disability	Elevated 4000-9000	Dystrophic	αDG	*POMT1* NM_007171.3	AR	c.132A>C, p.(Glu44Asp); c.2005G>A, p.(Ala669Thr) LOVD ID #54684	Parents heterozygous
M	8	WES	Onset age 1y, gross motor delay, walked age 2.5y, proximal weakness, choreiform movements, moderate intellectual disability, MRI - T2 hyperintensities	Elevated 600-3100	Non-specific	U	*MICU1* NM_006077.3	AR	c.386G>C; p.(Arg129Pro); c.1A>G LOVD ID #54685	Parents heterozygous
M	6	WES	Onset age 3mo, congenital hip dislocation, gross motor delay, unable to walk, mild facial weakness, mild scoliosis	Normal	Dystrophic	U	*RYR1* NM_000540.2	AD	c.13927G>C, p.(Ala4643Pro) LOVD ID #54686	De novo
F	11	CGS	Infantile hypotonia, omphalocoele, gross motor delay, walked age 2y, mild facial weakness, distal laxity, contractures,	Mild elevation 254	Dystrophic	COLVI	*COL6A1* NM_001848.2	AD	c.842G>A, p(.Gly281Glu) LOVD ID #54707	De novo
F	5	WES and WGS	Infantile onset, bilateral congenital femoral fractures, gross motor delay, walked age 4y, facial weakness, hypermobility, distal laxity, pectus excavatum, gastrostomy fed, mild sleep disordered breathing	Normal	Dystrophic	NA				
M	10	WES	Onset age 1y, hypotonia, oesophageal strictures, moderate intellectual disability	Normal	Non-specific	COLVI				
M	6	CGS	Infantile hypotonia, gross motor delay, stood with support age 3y,macrocephaly, moderate obstructive sleep apnoea	Mild elevation 884-930	Dystrophic	NA	*LMNA* NM_170707.3	AD	c.94_96delAGA, p.(Lys32del) LOVD ID #54687	De novo
M	20	WES	Infantile hypotonia, gross motor delay, walked age 2y, hypermobility and distal laxity, pectus excavatum, mild restrictive lung disease, mild learning difficulties	Normal	Non-specific	U				
F	5	WES and WGS	Onset age 6mo, congenital hip dysplasia, gross motor delay, not able to stand at 4y, mild facial weakness, hypermobility, distal laxity,	Normal	Non-specific	U				
F	5	CGS	Infantile hypotonia, arthrogryposis, congenital hip dysplasia, gross motor delay, contractures, hyperkeratosis pilaris, prominent heels, hypertrophic scars	Mild elevation 338	Dystrophic	COLVI	*COL6A1* NM_000088.3	AD	c.868G>A, p.(Gly290Arg) LOVD ID #54708	De novo
F	4	CGS	Infantile hypotonia, arthrogryposis, gross motor delay, contractures	Elevated 1650	Dystrophic	Laminin-α2	*LAMA2* NM_000426.2	AR	c.2556delT, p.(Phe852LeufsX36); c.3630delT, p.(Ile1210MetfsX14)	Parents heterozygous

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										3LOVD ID #54688	
13	M	3	WES	Infantile hypotonia, gross motor delay, walked age 2y9mo, facial weakness, hypermobility, distal laxity, obstructive sleep apnoea	Normal	Dystrophic	COLVI	*ACTA1* NM_001100.3	AD	c.493G>T; p.(Val165Leu) LOVD ID #54689	De novo
14	M	8	WES	Onset age 1y, gross motor delay, walked age 2y, mild facial weakness, distal laxity, mild speech delay	Normal	Non-specific	NA				
15	F	6	WES	Onset age 1y, hypotonia, proximal weakness, facial weakness, ophthalmoplegia, stroke-like episodes and seizures, respiratory weakness, tracheostomy, mild learning difficulties	Mild elevation 450-600	Dystrophic	U				
16	F	4	Panel B	Infantile hypotonia, gross motor delay, walked age 4y, contractures, prominent neck flexor weakness	Elevated 799-2470	NA	NA	*LMNA* NM_170707.2	AD	c.1151A>G (p.Glu384Gly) LOVD ID #54690	De novo
17	F	5	Panel B	Gross motor delay, standing at 4y	Elevated 1157	NA	NA	*LAMA2* NM_000426.2	AR	Hom.g.129608991G>C, c.2538-1G>C LOVD ID #54691	Parents heterozygous
18	F	9	CGS	Infantile hypotonia, gross motor delay, non-ambulant, distal laxity, contractures, scoliosis, hyperkeratosis	Mild elevation 415	NA	NA	*COL6A1* NM_001848.2	AD	c.868G>A, p.(Gly290Arg) LOVD ID #54709	De novo
19	M	3	CGS	Onset age 1y, gross motor delay, walked age 3.5y, hypermobility, distal laxity	Elevated 926-1310	NA	NA	*LMNA* NM_170707	AD	c.116A>G, p.(Asn39Ser) LOVD ID #54692	De novo
20	F	11		Onset age 6mo, proximal weakness, borderline intellectual disability	Elevated 5841	NA	NA				
21	M	10		Infantile onset, proximal and axial weakness, hypermobility, distal laxity, prominent heels, hyperkeratosis	Normal	NA	NA				
22	F	13	Panel A	Onset age 2y, proximal weakness, mild facial weakness, hypermobility, distal laxity, scoliosis, bifid uvula, macrocephaly, mild learning difficulties	Normal	Non-specific	U				
23	F	8	CGS	Infantile hypotonia, congenital hip dysplasia, gross motor delay, sat age 2y, contractures, nocturnal hypoventilation, MRI - white matter signal abnormality	Elevated 2500	Dystrophic	Laminin-α2	*LAMA2* NM_00426.2	AR	g.129807768G>A, c.7898+1G>A; c.8931_8933delTCT, p.(Leu2978del) LOVD ID #54693	Parents heterozygous

* The patient was part of the cohort published by Peat et al, Diagnosis and etiology of congenital muscular dystrophy, Neurology. 2008; 71:312-321.

[+] Age at which the patient died.

[z] Creatine kinase: Mild elevation = Normal for age – 1000; Elevated >1000

[U] Unconfirmed diagnosis: Patient 5 - RNA sequencing studies pending; Patient 33 and 71 - the clinical and IHC findings were consistent with the diagnosis, but only a heterozygous genetic variant was identified; Patient 93 - clinical findings were consistent with *TTN* myopathy. Two missense variants were maternally inherited, but classified as Ambry Class 3; Patient 96 – clinical findings consistent with *TTN* myopathy, but the maternal variant was classified as Ambry Class 3.

[a] Previously published by Clarke et al, Ann Neurol. 2006; 59:546-552.

[b] Previously published by Carrs et al, Am J Hum Gen. 2013; 93:29-41.

[c] Previously published by O'Grady et al, E J Hum Gen. 2015: 23:883-886.

[d] Previously published by Ilkovski et al, Hum Mol Gen. 2015: 24:6146-6159.

[e] Previously published by Susman et al, Expanding the clinical, pathological and MRI phenotype of *DNM2*-related centronuclear myopathy, Neuromuscular disorders. 2010; 20:229-237.

CGS, candidate generation sequencing; WES, whole exome sequencing, WGS, whole genome sequencing; CMA, chromosomal microarray; CK, creatine kinase; Uk, unknown; IHC, immunohistochemistry; U, unclassified; αDG, α-dystroglycanopathy; AR, autosomal recessive; AD, autosomal dominant; Hom., homozygous.

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Table e-2 Clinical and histological characteristics of patients with collagen VI, and *LMNA*-related CMD

Gene	Mutation	Patient ID, Sex, Mode of diagnosis, Clinical features	Pathogenicity Novel/previously reported variant
COL6A1 NM_001848.2 *COL6A2* NM_001849.3	c.793_804+9del21, g.47407557_474077del, p.(Pro254_Glu268del15); c.1970-3C>A, g.47545696C>A, p.(Gly657_Arg698del42)	100. M. CGS. Infantile hypotonia, weakness, gross motor delay, mild facial weakness, distal laxity, contractures, hyperkeratosis pilaris, finger flexor contractures. CK 419.	Novel; Reported. Confirmed de novo; paternally inherited. IHC - severe reduction in COLVI.
COL6A1 NM_001848.2	c.842G>A, p(.Gly281Glu)	105. F. CGS. Infantile hypotonia, omphalocoele, gross motor delay, mild facial weakness, distal laxity, contractures. CK 254.	Reported. Confirmed de novo. Phenotype consistent. IHC - moderate reduction in COLVI.
	c.868G>A, p.(Gly290Arg)	111. F. CGS. Hypotonia, weakness, arthrogryposis, congenital hip dysplasia, gross motor delay, contractures, hyperkeratosis pilaris, hypertrophic scarring. CK 338.	Reported. Confirmed de novo. Phenotype consistent. IHC - moderate reduction in COLVI.
	c.868G>A, p.(Gly290Arg)	118. F. CGS. Hypotonia, non-ambulant, distal laxity, contractures, scoliosis, hyperkeratosis pilaris. CK 415.	Reported. Confirmed de novo. Phenotype consistent. No IHC.
	c.877G>A p.(Gly293Arg)	58. M. CGS. Onset age 18mo, weakness, hypermobility and distal laxity. CK 265-306.	Reported. Confirmed de novo. IHC – mild reduction in COLVI
	c.887G>T, p.(Gly296Val)	62. F. CGS. Onset age 23mo, proximal weakness, distal laxity, hyperkeratosis pilaris. CK 395.	Reported. Confirmed de novo. IHC – severe reduction in COLVI
	c.1660delG, p.(Asp554ThrfsX48) (Hom.)	70. M. CGS. Infantile onset, gross motor delay, distal laxity, contractures, hyperkeratosis pilaris, restrictive lung disease. CK 245.	Novel. Parents heterozygous. Phenotype consistent. IHC - absent COLVI.
COL6A2 NM_001849.3	c.316G>A, p.(Glu106Lys); c.736_801del66, p.(Cys246_Lys267del22)	101. F. CGS. Infantile hypotonia, congenital hip dysplasia, talipes, weakness, hypermobility, distal laxity, mild scoliosis, hyperkeratosis pilaris. CK 343-405.	Reported; Novel. Paternally inherited missense variant and de novo deletion. Phenotype consistent. IHC -severe reduction in COLVI.
	c.785G>A, p.(Gly262Asp)	90. M. CGS. Onset age 6mo, weakness, mild facial weakness, hypermobility and distal laxity, contractures, hyperkeratosis pilaris. CK 375-603.	Novel. Confirmed de novo. Phenotype consistent. IHC - moderate reduction in COLVI.
	c.801+2T>C, g.47533989T>C,	68. F. CGS. Infantile hypotonia, congenital hip dysplasia, gross motor delay, mild facial weakness, distal laxity, scoliosis, hyperkeratosis pilaris, long finger flexor contractures. CK normal.	Novel. Confirmed de novo. Phenotype consistent. IHC - moderate reduction in COLVI.
	c.954G>T, p.(Lys318Asn)	26. F. Panel A. Onset age 6mo, non-ambulant, contractures, distal laxity. CK normal.	Reported. Confirmed de novo. Phenotype consistent. IHC - moderate reduction COLVI.
	c.1855_1860delGTCATC, p.(Val619_Ile620del); g.47545179G>T, c.1771-1G>T	69. F. CGS. Infantile hypotonia, arthrogryposis, congenital femur fracture and hip dislocation, gross motor delay, mild facial weakness, contractures, distal laxity, hyperkeratosis pilaris, restrictive lung disease. CK 378.	Novel; Novel. Parents heterozygous. Phenotype consistent. IHC - moderate reduction in COLVI.
COL6A3 NM_004369.3	c.6210+1 G>A, g.238269763C>T	23. M. CGS. Infantile hypotonia, congenital femur fracture, progressive weakness, distal laxity, hyperkeratosis pilaris, restrictive lung disease. CK 282.	Reported. Confirmed de novo. Phenotype consistent. IHC - severe reduction in COLVI.
	c.6221G>A, p.(Gly2074Asp)	85. M. CGS. Infantile hypotonia, arthrogryposis, congenital hip dislocation, gross motor delay, mild facial weakness, contractures, mild scoliosis. CK normal.	Reported. Confirmed de novo. Phenotype consistent. IHC - severe reduction in COLVI.
	c.6284 G>T p.(Gly2095Val)	63. M. CGS. Onset age 2y, congenital hip dislocation, proximal weakness, distal laxity, contractures. CK 475.	Novel. Confirmed de novo. IHC – moderate reduction in COLVI.
LMNA NM_170707.3	c.94_96delAGA, p.(Lys32del)	106. M. CGS. Infantile hypotonia, gross motor delay, macrocephaly, obstructive sleep apnoea. CK 884-930.	Reported. Confirmed de novo. Phenotype consistent.
	c.116A>G, p.(Asn39Ser)	92. F. CGS. Onset age 18mo, progressive weakness with loss of ambulation age 10y, contractures, tachyarrhythmia requiring pacemaker, restrictive lung disease. CK 650-900.	Reported. Confirmed de novo. Phenotype consistent.
	c.116A>G, p.(Asn39Ser)	119. M. CGS. Onset age 1y, gross motor delay, hypermobility, distal laxity. CK 926-1310.	Reported. Confirmed de novo. Phenotype consistent.
	c.810G>T, p.(Lys270Asn)	47. F. CGS. Onset age 16mo, progressive weakness with loss of ambulation at 10y, contractures, scoliosis, restrictive lung disease. CK 448.	Reported. Confirmed de novo. Phenotype consistent.
	c.1151A>G (p.Glu384Gly)	116. M. Panel B. Infantile hypotonia, gross motor delay, contractures, neck flexor weakness. CK 799-2470.	Novel. Confirmed de novo. Phenotype consistent.
	c.1366A>G, p.(Asn456Asp)	16. M. CGS. Onset age 6mo, gross motor delay, progressive weakness with loss of ambulation in teenage years, mild facial weakness. CK 863-1710.	Reported. Confirmed de novo. Phenotype consistent.

Further information including Leiden open variant database ID# is available in Supplementation Table-e1.

F, female; M, Male; CGS, candidate generation sequencing; CK, creatine kinase; IHC, immunohistochemistry; αDG, α-dystroglycanopathy; Hom., homozygous; Bx., biopsy.

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Table e-3 Clinical and histological characteristics of patients with α-dystroglycanopathies, laminin-α deficient and SEPN1-related CMD

Gene	Mutation	Patient ID, Sex, Mode of diagnosis, Clinical features	Pathogenicity Novel/previously reported variant
FKRP NM_024301.2	c.826C>A, p.(Leu276Ile); c.162_165dupGGAG, p.(Phe56GlyfsX6)	59. F. CGS. Gross motor delay, mild facial weakness, distal laxity, scoliosis. Bx. dystrophic. CK 4100.	Reported. Parents heterozygous. Phenotype, CK and Bx. consistent. IHC – αDG severely reduced.
FKTN NM_001079802.1	c.340G>A, p.(Ala114Thr); c.859delA, p.(Thr286fs)	45. F. CGS. Onset age 8mo, congenital hip dysplasia, gross motor delay, contractures. Bx. dystrophic. CK 5735-9000.	Reported. Parents heterozygous. Phenotype, CK and Bx. consistent. IHC – αDG severely reduced.
POMT1 NM_007171.3	c.132A>C, p.(Glu44Asp); c.2005G>A, p.(Ala669Thr)	102. F. WES. Onset age 18mo, gross motor delay, contractures, hepatosplenomegaly, moderate intellectual disability. Bx dystrophic. CK 4000-9000	Novel; Reported. Parents heterozygous. Phenotype, CK and Bx. consistent. IHC - moderate reduction in αDG.
	c.1847_1849delGGT, p.(Trp616del); c.193G>A, p.(Gly65Arg)	61. M. CGS. Hypotonia and gross motor delay, proximal weakness, MRI brain - white matter abn. Bx dystrophic. CK 3275-7806.	Reported; Novel. Parents heterozygous. Phenotype and CK consistent. IHC – absent αDG.
POMT2 NM_013382.5	c.1238G>C, p.(Arg413Pro); c.1997A>G, p.(Tyr666Cys)	79. M. CGS. Infantile weakness, contractures, White matter abn, cerebellar cysts. Bx dystrophic. CK 6752.	Novel; Reported. Parents heterozygous. Inv. consistent. IHC - absent αDG.
	c.551C>T, p.(Thr184Met); c.2243G>C, p.(Trp748Ser);	12. M. CGS. Onset age 18mo, gross motor delay, mild cognitive delay. Bx dystrophic. CK 1453-1894.	Reported; Novel. Parents heterozygous. Phenotype, CK and Bx. consistent. IHC - moderate reduction in αDG.
POMGNT1 NM_001243766.1	c.1100G>A, p.(Arg367His); c.1539+1G>A; g.46657769C>T	38. F. CGS. Onset age 6mo, gross motor delay, moderate cognitive delay, seizures, MRI brain - Walker Warburg syndrome. CK 996-2824.	Novel; Reported. Parents heterozygous. Phenotype, MRI and Bx. consistent. IHC - moderate reduction αDG
LARGE NM_004737.4	c.1640G>A, p.(Arg547His); Intragenic deletion (22q12.3(33,774,511-34,221,251)	98. M. WES and CMA. Infantile hypotonia, gross motor delay, MRI – abn. white matter. CK elevated.	Novel. Parents heterozygous. Phenotype and MRI brain consistent. IHC - absent αDG.
	c1640G>A, p.(Arg547His) + ?	33. F. CGS. Onset 1y, gross motor delay, moderate cognitive delay, MRI brain - leukodystrophy. CK 5000-7000.	Phenotype, MRI and CK consistent. IHC - absent αDG. CMA recommended to look for intragenic deletion.
GMPPB NM_013334.3	c.79G>C, (p.Asp27His); c.1036C>A, (p.Arg346Ser)	20. F. Panel B. Onset age 6mo, gross motor delay, moderate cognitive delay, choreoathetosis, rhabdomyolysis. CK 943-6728.	Novel. Parents heterozygous. Phenotype consistent. IHC – mild reduction in αDG. Published[a].
	c.553C>T, p.(Arg185Cys) (Hom.)	31. M. WES. Onset age 2y, gross motor delay, cataracts, mild cognitive delay, seizures. CK 4344-5200.	Novel. Parents heterozygous. IHC - severe reduction in αDG. Published[b].
LAMA2 NM_000426.2	c.2538-1G>C, g.129608991G>C, (Hom.)	117. F. Panel B. Gross motor delay, standing at 4y. CK 1157.	Reported. Parents heterozygous. Phenotype and CK consistent. No Bx.
	c.2556delT, p.(Phe852LeufsX36); c.6919_6920delTA, p.(Tyr2307LeufsX2)	95. M. CGS. Infantile hypotonia and weakness, arthrogryposis, congenital hip dysplasia, gross motor delay, contractures. Bx. dystrophic. CK 2600.	Reported; Novel. Parents heterozygous. Phenotype, CK and histology consistent. IHC - absent laminin-α2
	c.2556delT, p.(Phe852LeufsX36); c.3630delT, p.(Ile1210MetfsX14)	112. F. CGS. Infantile hypotonia, arthrogryposis, gross motor delay, contractures. CK 1650.	Reported. Parents heterozygous. IHC - absent laminin-α2.
	c.4523G>C, p.(Arg1508Thr); c.4645C>T, p.(Arg1549X)	51. F. CGS. Onset age 3mo, gross motor delay, contractures, nocturnal ventilation, MRI - cobblestone lissencephaly. CK 487-3260.	Reported. Parents heterozygous. Phenotype, CK, and MRI consistent. IHC - severe reduction in laminin-α2.
	c.4860G>A, p.(Phe1573Ser fs49X); + ?	71. M. CGS. Infantile onset, gross motor delay, walked at 4 years, contractures, spinal rigidity. CK normal.	Novel. Paternally inherited. No second variant identified. IHC – moderate reduction in laminin- α2, but CK normal.
	c.7691T>C, p.(Leu2564Pro); c.9253C>T, p.(Arg3085X)	84. M. Panel A. Onset age 6mo, proximal weakness, mild scoliosis. Bx. dystrophic. CK 3195.	Reported. Parents heterozygous. IHC - absent laminin-α2
	c.7898+1G>A, g.129807768G>A; c.8931_8933delTCT, p.(Leu2978del)	123. F. CGS. Infantile hypotonia, congenital hip dysplasia, gross motor delay, contractures. Leukodystrophy on MRI. Bx. dystrophic. CK 2500.	Novel. Segregation confirmed in parents and affected sibs. Inv. consistent. IHC - moderate reduction in laminin-α2.
	Report not available	28. F. CGS. Onset age 6mo, gross motor delay, mild facial weakness, scoliosis, cognitive delay, MRI brain T2 white matter hyperintensities. CK 2500.	Phenotype, CK, histology and brain. MRI consistent. IHC - moderate reduction laminin-α2.
SEPN1 NM_020451.2	c.943G>A, p.(Gly315Ser) (Hom.)	1. F. CGS. Onset age 8mo, gross motor delay, later loss of ambulation, scoliosis. Bx. mildly dystrophic. CK normal.	Reported. Parents heterozygous, affected sibling hom. Published[c].
	c.1397G>A, p.(Arg466Gln) (Hom).	49. M. CGS. Onset age 6mo, progressive course with loss of ambulation from 10y, mild facial weakness, scoliosis, restrictive lung disease. CK 57-503.	Reported. Parents heterozygous. Phenotype consistent.

Further information including Leiden open variant database ID# is available in Supplementation Table-e1.

The following patients have been published elsewhere: [a] 20 – Cabrera-Serrano et al, Brain. 2015;138;836-844 (Family V); [b] 31 – Carrs et al, Am J Hum Gen. 2013; 93:29-41 (P8); [c] 1 – Clarke et al, Ann Neurol. 2006; 59:546-552 (Patient 1);

F, female; M, Male; Hom., homozygous; CGS, candidate generation sequencing; WES, whole exome sequencing; CMA, chromosomal microarray; Bx, Biopsy; CK, creatine kinase; IHC, immunohistochemistry; Inv., Investigations.

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Table e-4 Clinical and histological characteristics of patients with other genetic diagnoses

Gene	Mutation	Patient ID, Sex, Mode of diagnosis, Clinical features	Pathogenicity Novel/previously reported variant
ACTA1 NM_001100.3	Hom. c.460G>C, p.(Val154Leu)	32. M. Panel A. Onset age 2y, gross motor delay, slowly progressive weakness, contractures, scoliosis, spinal rigidity. Bx. dystrophic. CK 396-1930.	Novel. Parents heterozygous, affected brother hom. Novel phenotype – published[a].
	c.493G>T; p.(Val165Leu)	113. M. WES. Infantile hypotonia, gross motor delay, facial weakness, hypermobility, distal laxity. CK normal.	Reported. Confirmed de novo. Phenotype consistent. H&E and EM consistent with intranuclear nemaline rods.
DNM2 NM_001005360.2	c.1102G>A, p.(Glu368Lys)	66. F. CGS Infantile onset, gross motor delay, proximal weakness, facial weakness, ptosis, ophthalmoplegia, contractures. CK normal. Bx. Increased central nuclei.	Reported. Confirmed de novo. Consistent with expanded phenotype of *DNM2*-related disease. Published[b].
	c.1880C>A, p.(Pro627His)	56. M. CGS. Infantile hypotonia, eventration of diaphragm, gross motor delay, facial weakness, ptosis, ophthalmoplegia, contractures. CK normal. Bx. dystrophic.	Novel. Confirmed de novo. Phenotype consistent.
GFPT1 NM_001244710.1	c.686-2A>G, g.69581446T>C; c.1072A>G, p.(Met358Val)	60. F. WES. Congenital hypotonia, gross motor delay, mild facial weakness, contractures, scoliosis. CK normal. Bx dystrophic with degenerating and regenerating fibres.	Novel. Parents heterozygous. EPS studies confirm neuromuscular junction dysfunction. Response to pyridostigmine.
MICU1 NM_006077.3	c.386G>C, p.(Arg129Pro); c.1A>G	103. M. WES. Onset age 1y, gross motor delay, proximal weakness, choreiform movements, moderate intellectual disability, MRI - T2 hyperintensities. CK 600-3100.	Novel. Parents heterozygous. Phenotype, with extrapyramidal movement disorder, and CK consistent.
PIGY NM_001042616	c.137T>C, p.(Leu46Pro) (Hom.)	44. F. WES. Infantile hypotonia, profound global delay, arthrogryposis, seizures. Bx. dystrophic. CK 323-554.	Novel. Parents heterozygous, affected sister hom. Functional studies consistent. Published[c].
RYR1 NM_000540.2	c.270+2T>C, g.38933095T>C; c.6721C>T, p.(Arg2241X)	5. M. WES. Infantile hypotonia and gross motor delay, mild facial weakness, ptosis, contractures, scoliosis, biventricular impairment, restrictive lung disease. Bx – increased internalized nuclei. Cores on repeat bx. at 10y.	Novel; reported. Parents heterozygous. Phenotype and histology consistent. Unconfirmed diagnosis - cDNA studies to assess splice site variant pending.
	c.6443C>T, p.(Ser2148Phe); c. 7361G>A, p.(Arg2454His)	41. M. CGS. Infantile onset, congenital hip dysplasia, gross motor delay, facial weakness. Bx. dystrophic – variation in fibre size, internalized nuclei, degenerative change. CK normal. Sparing of rectus femoris on muscle MRI	Novel. Parents heterozygous. Phenotype consistent. Muscle MRI typical. Dystrophic findings reported in *RYR1*-related myopathy.
	c.6721C>T, p.(Arg2241X); c.8693-124T>G, g.38997345T>G, p.(Gly2898Aspfs*15)	46. M. CGS. Infantile hypotonia, gross motor delay, non-ambulant from 11y, facial weakness, ophthalmoplegia, contractures, scoliosis. CK normal. Bx. Variation in fibre size, internalized nuclei, fibrosis, fatty infiltrate.	Reported; novel. Parents heterozygous. cDNA analysis: Intronic variant creates a donor site and activates an acceptor site for a cryptic exon, causing frameshift.
	c.13927G>C, p.(Ala4643Pro)	104. M. WES. Congenital hip dysplasia, gross motor delay, non-ambulant, mild facial weakness, scoliosis. Bx. dystrophic, increased central nuclei. CK normal. Muscle MRI – rectus femoris sparing.	Novel. Confirmed de novo. Phenotype and muscle MRI consistent.
SIL1 NM_001037633.1	c.178G>T, p.(Glu60X); c.346delG (p.Gly116AlafsX42)	36. M. CGS. Onset age 6mo, gross motor delay, ataxia, congenital cataracts, cerebellar hypoplasia and atrophy. CK 215-309.	Novel. Parents heterozygous, affected brother compound heterozygous. Phenotype and MRI consistent.
TTN NM_001267550.1	c. 42315_42318del, p.(Lys14105Asnfs), g.179499190_179499193delGAAA; c. 67349-2A>C, g.179444577T>G	91. M. Panel A. Infantile hypotonia and weakness, mild facial weakness, ptosis, contractures, dilated cardiomyopathy in 20s, cardiac arrest. CK normal. Bx. Type 1 fibre predominance.	Novel. C.67349-2A>C maternally inherited. C.42315_42318del presumed paternally inherited (deceased). Phenotype consistent.
	c.25335dup, p.(Lys8446GlnfsX8); c.45328G>A, p.(Asp15110Asn) and c.23177C>T, p.(Ser7726Leu)	93. M. WES and WGS. Infantile hypotonia, arthrogryposis, congenital fractures, gross motor delay, mild facial weakness, distal laxity, contractures, scoliosis. CK normal. Bx. Extensive fibro-fatty infiltrate.	Clinical findings and histology consistent. Paternally inherited truncating variant. Pathogenicity of maternally inherited missense variants unable to be confirmed.
	g.179466399del, c.55418delG, p.(Arg18473fsX14); g.179433110_179433127del, c.77732_77749del, p.(Ser25911_Leu25916del)	96. M. Panel A and Panel B. Infantile onset, gross motor delay, facial weakness, distal laxity, contractures, scoliosis, rigid spine, dilated cardiomyopathy, restrictive lung disease. CK normal. Bx. dystrophic with multiple internalized nuclei.	Phenotype and histology consistent. Paternally inherited truncating variant. Pathogenicity of maternally inherited deletion unable to be confirmed.
Microdeletion (CMA)	14q13.2-q21.1 (35,327,739-38,602,335)	77. M. CMA. Infantile hypotonia, gross motor delay, distal laxity and hypermobility, contractures, extrapyramidal movements from 18mo. CK normal. Bx. non-specific.	Confirmed de novo. Includes *NKX2-1*. Later development of extrapyramidal movements consistent with benign hereditary chorea.
Microdeletion (CMA)	6q13-q14.1 (75,540,881 - 83,110,141) incl *COL12A1*	81. M. CMA. Infantile hypotonia, arthrogryposis, congenital hip dysplasia, gross motor delay, contractures, hypermobility, scoliosis. CK normal.	Confirmed de novo. Phenotype consistent with *COL12A1* deletion.

Further information and Leiden open variant database ID# is available in Supplementation Table-e1.

The following patients have been published elsewhere: [a] 32 – O'Grady et al, E J Hum Gen. 2015:23;883-886 [b] 66 - Susman et al, Neuromuscular disorders. 2010:20;229-237 (Patient 4); [c] 44 - Ilkovski et al, Hum Mol Gen. 2015: 24;6146-6159 (Family A).

F, female; M, Male; CGS, candidate generation sequencing; WES, whole exome sequencing; WGS, whole genome sequencing; CMA, chromosomal microarray; CK, creatine kinase; Hom., homozygous; H&E, Haemotoxylin and eosin; EM, electron microscopy; EPS, electrophysiological.

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Table e-5 Genes included in Panel A and Panel B

Panel A

Gene Name	HGNC_ID	Description	Gene Name	HGNC_ID	Description
ACTA1	129	Skeletal alpha actin 1	MATR3	6912	Matrin-3
BAG3	939	BCL2-associated athanogene	MTM1	7448	Myotubularin
CAPN3	1480	Calpain 3, (p94)	MYH7	7577	Slow/ beta cardiac myosin heavy chain
CAV3	1529	Caveolin 3	MYOT	12399	Myotilin
CFL2	1875	Cofilin 2	NEB	7720	Nebulin
COL6A1	2211	Collagen, type VI, alpha 1	POMGNT1	19139	Protein O-linked mannose beta1,2-N-acetylglucosaminyltransferase
COL6A2	2212	Collagen, type VI, alpha 2	POMT1	9202	Protein-O-mannosyltransferase 1
COL6A3	2213	Collagen, type VI, alpha 3	POMT2	19743	Protein-O-mannosyltransferase 2
CRYAB	2389	AlphaB crystallin	RYR1	10483	Ryanodine receptor 1 (skeletal)
DES	2770	Desmin	SEPN1	15999	selenoprotein N,1
DMD	2928	Dystrophin (Duchenne and Becker types)	SGCA	10805	sarcoglycan, alpha (50kDa dystrophin-associated glycoprotein)
DNM2	2974	Dynamin 2	SGCB	10806	sarcoglycan, beta (43kDa dystrophin-associated glycoprotein)
DYSF	3097	Dysferlin, limb girdle muscular dystrophy 2B	SGCD	10807	sarcoglycan, delta (35kDa dystrophin-associated glycoprotein)
EMD	3331	Emerin (Emery-Dreifuss muscular dystrophy)	SGCG	6445	Sarcoglycan, gamma
FHL1	3702	Four and a half LIM domains 1	SMN1	11117	Survival of motor neuron 1, telomeric
FKRP	17997	Fukutin related protein	TCAP	11610	Titin-cap
FKTN	3622	Fukutin	TPM2	12011	tropomyosin 2 (beta)
FLNA	3754	Filamin A	TPM3	12012	Tropomyosin 3
GAA	842	Acid alpha-glucosidase preproprotein	TRIM32	16380	Tripartite motif-containing 32
ITGA7	6143	Integrin, alpha 7	TTN	12403	Titin
LAMA2	6482	Laminin, alpha 2 (merosin)	VCP	12666	Valosin containing protein
LARGE	6511	Like-glycosyltransferase			
LDB3	15710	LIM domain binding 3			
LMNA	6636	Lamin A/C			

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Panel B

Gene Name	HGNC_ID	Description	Gene Name	HGNC_ID	Description
AARS	20	Alanyl-tRNA synthetase	KIF21A	19329	kinesin family member 21A
ABCC9	60	ATP-binding cassette, sub-family C (CFTR/MRP), member 9	KIF5A	6323	kinesin family member 5A
ABCD1	61	ATP-binding cassette, sub-family D (ALD), member 1	KLHL9	18732	kelch-like family member 9
ABHD12	15868	abhydrolase domain containing 12	L1CAM	6470	L1 cell adhesion molecule
ABHD5	21396	abhydrolase domain containing 5	LAMA2	6482	laminin, alpha 2
ACADVL	92	acyl-CoA dehydrogenase, very long chain	LAMA4	6484	laminin, alpha 4
ACTA1	129	actin, alpha 1, skeletal muscle	LAMB2	6487	laminin, beta 2 (laminin S)
ACTC1	143	actin, alpha, cardiac muscle 1	LAMP2	6501	lysosomal-associated membrane protein 2
ACTN2	164	actinin, alpha 2	LARGE	6511	like-glycosyltransferase
ACVR1	171	activin A receptor, type I	LDB3	15710	LIM domain binding 3
ADCK3	16812	aarF domain containing kinase 3	LDHA	6535	lactate dehydrogenase A
AFG3L2	315	AFG3-like AAA ATPase 2	LITAF	16841	lipopolysaccharide-induced TNF factor
AGL	321	amylo-alpha-1, 6-glucosidase, 4-alpha-glucanotransferase	LMNA	6636	lamin A/C
AGRN	329	agrin	LMOD3	6649	leiomodin 3 (fetal)
AHNAK	347	AHNAK nucleoprotein	LPIN1	13345	lipin 1
AIFM1	8768	apoptosis-inducing factor, mitochondrion-associated, 1	LRSAM1	25135	leucine rich repeat and sterile alpha motif containing 1
ALDH3A2	403	aldehyde dehydrogenase 3 family, member A2	MATR3	6912	matrin 3
ALS2	443	amyotrophic lateral sclerosis 2 (juvenile)	MED25	28845	mediator complex subunit 25
ANG	483	angiogenin, ribonuclease, RNase A family, 5	MEGF10	29634	multiple EGF-like-domains 10
ANK2	493	ankyrin 2, neuronal	MFN2	16877	mitofusin 2
ANKRD1	15819	ankyrin repeat domain 1 (cardiac muscle)	MPZ	7225	myelin protein zero
ANO10	25519	anoctamin 10	MRE11A	7230	MRE11 homolog A, double strand break repair nuclease
ANO5	27337	anoctamin 5	MRPL3	10379	mitochondrial ribosomal protein L3
AP5Z1	22197	adaptor-related protein complex 5, zeta 1 subunit	MSTN	4223	myostatin
			MTM1	7448	myotubularin 1
APOA1	600	apolipoprotein A-I	MTMR2	7450	myotubularin related protein 2
APTX	15984	aprataxin	MTTP	7467	microsomal triglyceride transfer protein
AR	644	androgen receptor	MURC	33742	muscle-related coiled-coil protein

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ARHGEF10	14103	Rho guanine nucleotide exchange factor (GEF) 10	MUSK	7525	muscle, skeletal, receptor tyrosine kinase
ARSA	713	arylsulfatase A	MYBPC1	7549	myosin binding protein C, slow type
ARX	18060	aristaless related homeobox	MYBPC3	7551	myosin binding protein C, cardiac
ASAH1	735	N-acylsphingosine amidohydrolase (acid ceramidase) 1	MYH2	7572	myosin, heavy chain 2, skeletal muscle, adult
ATL1	11231	atlastin GTPase 1	MYH3	7573	myosin, heavy chain 3, skeletal muscle, embryonic
ATM	795	ATM serine/threonine kinase	MYH6	7576	myosin, heavy chain 6, cardiac muscle, alpha
ATP2A1	811	ATPase, Ca++ transporting, cardiac muscle, fast twitch 1	MYH7	7577	myosin, heavy chain 7, cardiac muscle, beta
ATP2B3	816	ATPase, Ca++ transporting, plasma membrane 3	MYH8	7578	myosin, heavy chain 8, skeletal muscle, perinatal
ATP7A	869	ATPase, Cu++ transporting, alpha polypeptide	MYL2	7583	myosin, light chain 2, regulatory, cardiac, slow
B3GALNT2	28596	beta-1,3-N-acetylgalactosaminyltransferase 2	MYL3	7584	myosin, light chain 3, alkali; ventricular, skeletal, slow
BAG3	939	BCL2-associated athanogene 3	MYLK2	16243	myosin light chain kinase 2
BEAN1	24160	brain expressed, associated with NEDD4, 1	MYOT	12399	myotilin
BIN1	1052	bridging integrator 1	MYOZ2	1330	myozenin 2
BSCL2	15832	Berardinelli-Seip congenital lipodystrophy 2 (seipin)	MYPN	23246	myopalladin
C10orf2	1160	chromosome 10 open reading frame 2	NDRG1	7679	N-myc downstream regulated 1
CACNA1A	1388	calcium channel, voltage-dependent, P/Q type, alpha 1A subunit	NDUFAF1	18828	NADH dehydrogenase (ubiquinone) complex I, assembly factor 1
CACNA1C	1390	calcium channel, voltage-dependent, L type, alpha 1C subunit	NEB	7720	nebulin
CACNA1S	1397	calcium channel, voltage-dependent, L type, alpha 1S subunit	NEFL	7739	neurofilament, light polypeptide
CACNB2	1402	calcium channel, voltage-dependent, beta 2 subunit	NEXN	29557	nexilin (F actin binding protein)
CACNB4	1404	calcium channel, voltage-dependent, beta 4 subunit	NGF	7808	nerve growth factor (beta polypeptide)
CAPN3	1480	calpain 3	NIPA1	17043	non imprinted in Prader-Willi/Angelman syndrome 1
CASQ2	1513	calsequestrin 2 (cardiac muscle)	NOTCH3	7883	notch 3
CAV3	1529	caveolin 3	NPPA	7939	natriuretic peptide A
CCT5	1618	chaperonin containing TCP1, subunit 5 (epsilon)	NTRK1	8031	neurotrophic tyrosine kinase, receptor, type 1
CFL2	1875	cofilin 2 (muscle)	OPA1	8140	optic atrophy 1 (autosomal dominant)
CHAT	1912	choline O-acetyltransferase	PABPN1	8565	poly(A) binding protein, nuclear 1
CHRNA1	1955	cholinergic receptor, nicotinic, alpha 1 (muscle)	PAFAH1B1	8574	platelet-activating factor acetylhydrolase 1b, regulatory subunit 1 (45kDa)
CHRNB1	1961	cholinergic receptor, nicotinic, beta 1 (muscle)	PDK3	8811	pyruvate dehydrogenase kinase, isozyme 3
CHRND	1965	cholinergic receptor, nicotinic, delta (muscle)	PEX7	8860	peroxisomal biogenesis factor 7
CHRNE	1966	cholinergic receptor, nicotinic, epsilon (muscle)	PFKM	8877	phosphofructokinase, muscle

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CHRNG	1967	cholinergic receptor, nicotinic, gamma (muscle)	PFN1	8881	profilin 1
CLCN1	2019	chloride channel, voltage-sensitive 1	PGAM2	8889	phosphoglycerate mutase 2 (muscle)
CNBP	13164	CCHC-type zinc finger, nucleic acid binding protein	PGK1	8896	phosphoglycerate kinase 1
CNTN1	2171	contactin 1	PGM1	8905	phosphoglucomutase 1
COL6A1	2211	collagen, type VI, alpha 1	PHKA1	8925	phosphorylase kinase, alpha 1 (muscle)
COL6A2	2212	collagen, type VI, alpha 2	PHOX2A	691	paired-like homeobox 2a
COL6A3	2213	collagen, type VI, alpha 3	PHYH	8940	phytanoyl-CoA 2-hydroxylase
COLQ	2226	collagen-like tail subunit (single strand of homotrimer) of asymmetric acetylcholinesterase	PIP5K1C	8996	phosphatidylinositol-4-phosphate 5-kinase, type I, gamma
COX15	2263	cytochrome c oxidase assembly homolog 15 (yeast)	PKP2	9024	plakophilin 2
CPT1B	2329	carnitine palmitoyltransferase 1B (muscle)	PLEC	9069	plectin
CPT2	2330	carnitine palmitoyltransferase 2	PLEKHG5	29105	pleckstrin homology domain containing, family G (with RhoGef domain) member 5
CRYAB	2389	crystallin, alpha B	PLN	9080	phospholamban
CSRP3	2372	mediator complex subunit 23	PLP1	9086	proteolipid protein 1
CTDP1	2498	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) phosphatase, subunit 1	PMP22	9118	peripheral myelin protein 22
CYP7B1	2652	cytochrome P450, family 7, subfamily B, polypeptide 1	PNPLA2	30802	patatin-like phospholipase domain containing 2
DAG1	2666	dystroglycan 1 (dystrophin-associated glycoprotein 1)	PNPLA6	16268	patatin-like phospholipase domain containing 6
DCTN1	2711	dynactin 1	POLG	9179	polymerase (DNA directed), gamma
DCX	2714	doublecortin	POLG2	9180	polymerase (DNA directed), gamma 2, accessory subunit
DES	2770	desmin	POMGNT1	19139	protein O-linked mannose N-acetylglucosaminyltransferase 1 (beta 1,2-)
DHTKD1	23537	dehydrogenase E1 and transketolase domain containing 1	POMT1	9202	protein-O-mannosyltransferase 1
DMD	2928	dystrophin	POMT2	19743	protein-O-mannosyltransferase 2
DMPK	2933	dystrophia myotonica-protein kinase	PRKAG2	9386	protein kinase, AMP-activated, gamma 2 non-catalytic subunit
DNAJB2	5228	DnaJ (Hsp40) homolog, subfamily B, member 2	PRKCG	9382	protein kinase, cAMP-dependent, catalytic, gamma
DNAJB6	14888	DnaJ (Hsp40) homolog, subfamily B, member 6	PRPS1	9462	phosphoribosyl pyrophosphate synthetase 1
DNM2	2974	dynamin 2	PRRT2	30500	proline-rich transmembrane protein 2
DNMT1	2976	DNA (cytosine-5-)-methyltransferase 1	PRX	13797	periaxin
DOCK3	2989	dedicator of cytokinesis 3	PSEN1	9508	presenilin 1
DOK7	26594	docking protein 7	PSEN2	9509	presenilin 2
DPAGT1	2995	dolichyl-phosphate (UDP-N-acetylglucosamine) N-acetylglucosaminephosphotransferase 1 (GlcNAc-1-P transferase)	PTRF	9688	polymerase I and transcript release factor
DPM2	3006	dolichyl-phosphate mannosyltransferase polypeptide 2, regulatory subunit	PYGM	9726	phosphorylase, glycogen, muscle

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DSC2	3036	desmocollin 2	RAB7A	9788	RAB7A, member RAS oncogene family
DSG2	3049	desmoglein 2	RAPSN	9863	receptor-associated protein of the synapse
DSP	3052	desmoplakin	RBM20	27424	RNA binding motif protein 20
DTNA	3057	dystrobrevin, alpha	REEP1	25786	receptor accessory protein 1
DYNC1H1	2961	dynein, cytoplasmic 1, heavy chain 1	RELN	9957	reelin
DYSF	3097	dysferlin	RRM2B	17296	ribonucleotide reductase M2 B (TP53 inducible)
EGR2	3239	early growth response 2	RYR1	10483	ryanodine receptor 1 (skeletal)
EMD	3331	emerin	RYR2	10484	ryanodine receptor 2 (cardiac)
ENO3	3354	enolase 3 (beta, muscle)	SACS	10519	sacsin molecular chaperone
ERBB3	3431	erb-b2 receptor tyrosine kinase 3	SBF2	2135	SET binding factor 2
ETFA	3481	electron-transfer-flavoprotein, alpha polypeptide	SCN4A	10591	sodium channel, voltage gated, type IV alpha subunit
ETFB	3482	electron-transfer-flavoprotein, beta polypeptide	SCN5A	10593	sodium channel, voltage gated, type V alpha subunit
ETFDH	3483	electron-transferring-flavoprotein dehydrogenase	SDHA	10680	succinate dehydrogenase complex, subunit A, flavoprotein (Fp)
EYA4	3522	EYA transcriptional coactivator and phosphatase 4	SEPN1	15999	selenoprotein N, 1
FA2H	21197	fatty acid 2-hydroxylase	SEPT9	7323	septin 9
FAM134B	25964	family with sequence similarity 134, member B	SETX	445	senataxin
FBLN5	3602	Fibulin 5	SGCA	10805	arcoglycan, alpha (50kDa dystrophin-associated glycoprotein)
FGD4	19125	FYVE, RhoGEF and PH domain containing 4	SGCB	10806	sarcoglycan, beta (43kDa dystrophin-associated glycoprotein)
FGF14	3671	fibroblast growth factor 14	SGCD	10807	sarcoglycan, delta (35kDa dystrophin-associated glycoprotein)
FGFR2	3689	fibroblast growth factor receptor 2	SGCE	10808	sarcoglycan, epsilon
FHL1	3702	four and a half LIM domains 1	SGCG	10809	sarcoglycan, gamma (35kDa dystrophin-associated glycoprotein)
FIG4	16873	FIG4 phosphoinositide 5-phosphatase	SH3TC2	29427	SH3 domain and tetratricopeptide repeats 2
FKRP	17997	fukutin related protein	SIL1	24624	SIL1 nucleotide exchange factor
FKTN	3622	fukutin	SLC12A6	10914	solute carrier family 12 (potassium/chloride transporter), member 6
FLNA	3754	filamin A, alpha	SLC1A3	10941	solute carrier family 1 (glial high affinity glutamate transporter), member 3
FLNC	3756	filamin C, gamma	SLC22A5	10969	solute carrier family 22 (organic cation/carnitine transporter), member 5
FUS	4010	FUS RNA binding protein	SLC25A20	1421	solute carrier family 25 (carnitine/acylcarnitine translocase), member 20
FXN	3951	frataxin	SLC25A4	10990	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4
GAA	842	glucosidase, alpha; acid	SLC33A1	95	solute carrier family 33 (acetyl-CoA transporter), member 1

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GAN	4137	gigaxonin	SMCHD1	29090	structural maintenance of chromosomes flexible hinge domain containing 1
GARS	4162	glycyl-tRNA synthetase	SMN1	11117	survival of motor neuron 1, telomeric
GATAD1	29941	GATA zinc finger domain containing 1	SOD1	11179	superoxide dismutase 1, soluble
GBE1	4180	glucan (1,4-alpha-), branching enzyme 1	SOX10	11190	SRY-box 10
GDAP1	15968	ganglioside induced differentiation associated protein 1	SPAST	11233	spastin
GFPT1	4241	glutamine--fructose-6-phosphate transaminase 1	SPG11	11226	pastic paraplegia 11 (autosomal recessive)
GJA5	4279	gap junction protein, alpha 5, 40kDa	SPG20	18514	spastic paraplegia 20 (Troyer syndrome)
GJB1	4283	gap junction protein, beta 1, 32kDa	SPG21	20373	spastic paraplegia 21 (autosomal recessive, Mast syndrome)
GJB3	4285	gap junction protein, beta 3, 31kDa	SPG7	11237	spastic paraplegia 7 (pure and complicated autosomal recessive)
GLE1	4315	GLE1 RNA export mediator	SPTBN2	11276	spectrin, beta, non-erythrocytic 2
GMPPB	22932	GDP-mannose pyrophosphorylase B	SPTLC1	11277	serine palmitoyltransferase, long chain base subunit 1
GNE	23657	glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase	SPTLC2	11278	serine palmitoyltransferase, long chain base subunit 2
GPD1L	28956	glycerol-3-phosphate dehydrogenase 1-like	STIM1	11386	stromal interaction molecule 1
(GTDC2) POMGNT2	25902	protein O-linked mannose N-acetylglucosaminyltransferase 2 (beta 1,4-)	SUCLA2	11448	succinate-CoA ligase, ADP-forming, beta subunit
GYG1	4699	glycogenin 1	SYNE1	17089	spectrin repeat containing, nuclear envelope 1
GYS1	4706	glycogen synthase 1 (muscle)	SYNE2	17084	spectrin repeat containing, nuclear envelope 2
HARS	4816	histidyl-tRNA synthetase	TARDBP	11571	TAR DNA binding protein
HCN4	16882	hyperpolarization activated cyclic nucleotide gated potassium channel 4	TAZ	11577	tafazzin
HINT1	4912	histidine triad nucleotide binding protein 1	TCAP	11610	titin-cap
HK1	4922	hexokinase 1	TDP1	18884	tyrosyl-DNA phosphodiesterase 1
HOXD10	5133	homeobox D10	TFG	11758	TRK-fused gene
HSPB1	5246	heat shock 27kDa protein 1	TGFB3	11769	transforming growth factor beta 3
HSPB3	5248	heat shock 27kDa protein 3	TIA1	11802	TIA1 cytotoxic granule-associated RNA binding protein
HSPB8	30171	heat shock 22kDa protein 8	TK2	11831	thymidine kinase 2, mitochondrial
HSPD1	5261	heat shock 60kDa protein 1 (chaperonin)	TMEM43	28472	transmembrane protein 43
HSPG2	5273	heparan sulfate proteoglycan 2	TMPO	11875	thymopoietin
IFRD1	5456	interferon-related developmental regulator 1	TNNC1	11943	troponin C type 1 (slow)
IGHMBP2	5542	immunoglobulin mu binding protein 2	TNNI2	11946	troponin I type 2 (skeletal, fast)
IKBKAP	5959	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein	TNNI3	11947	troponin I type 3 (cardiac)
ILK	6040	integrin-linked kinase	TNNT1	11948	troponin T type 1 (skeletal, slow)

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INF2	23791	inverted formin, FH2 and WH2 domain containing	TNNT2	11949	troponin T type 2 (cardiac)
ISCU	29882	iron-sulfur cluster assembly enzyme	TNNT3	11950	troponin T type 3 (skeletal, fast)
ISPD	37276	isoprenoid synthase domain containing	TNPO3	17103	transportin 3
ITGA7	6143	integrin, alpha 7	TOR1A	3098	torsin family 1, member A (torsin A)
ITPR1	6180	inositol 1,4,5-trisphosphate receptor, type 1	TPM1	12010	tropomyosin 1 (alpha)
JPH2	14202	junctophilin 2	TPM2	12011	tropomyosin 2 (beta)
JUP	6207	junction plakoglobin	TPM3	12012	tropomyosin 3
KARS	6215	lysyl-tRNA synthetase	TRIM32	16380	tripartite motif containing 32
KBTBD10	16905	kelch-like family member 41	TRPV4	18083	transient receptor potential cation channel, subfamily V, member 4
KBTBD13	37227	kelch repeat and BTB (POZ) domain containing 13	TTBK2	19141	tau tubulin kinase 2
KLHL40	30372	kelch-like family member 40	TTN	12403	titin
KCNA1	6218	potassium channel, voltage gated shaker related subfamily A, member 1	TTPA	12404	tocopherol (alpha) transfer protein
KCNA5	6224	potassium channel, voltage gated shaker related subfamily A, member 5	TTR	12405	transthyretin
KCNC3	6235	potassium channel, voltage gated Shaw related subfamily C, member 3	TUBA1A	20766	tubulin, alpha 1a
KCNE1	6240	potassium channel, voltage gated subfamily E regulatory beta subunit 1	TUBB3	20772	tubulin, beta 3 class III
KCNE2	6242	potassium channel, voltage gated subfamily E regulatory beta subunit 2	UBA1	12469	ubiquitin-like modifier activating enzyme 1
KCNE3	6243	potassium channel, voltage gated subfamily E regulatory beta subunit 3	UTRN	12635	utrophin
KCNH2	6251	potassium channel, voltage gated eag related subfamily H, member 2	VAPB	12649	VAMP (vesicle-associated membrane protein)-associated protein B and C
KCNJ12	6258	potassium channel, inwardly rectifying subfamily J, member 12	VCL	12665	vinculin
KCNJ18	39080	potassium channel, inwardly rectifying subfamily J, member 18	VCP	12666	Valosin containing protein
KCNJ2	6263	potassium channel, inwardly rectifying subfamily J, member 2	VMA21	22082	VMA21 vacuolar H+-ATPase homolog (S. cerevisiae)
KCNQ1	6294	potassium channel, voltage gated KQT-like subfamily Q, member 1	VRK1	12718	vaccinia related kinase 1
KIAA0196	28984	KIAA0196	WNK1	14540	WNK lysine deficient protein kinase 1
KIF1A	888	kinesin family member 1A	YARS	12840	tyrosyl-tRNA synthetase
KIF1B	16636	kinesin family member 1B	ZFYVE26	20761	zinc finger, FYVE domain containing 26
			ZFYVE27	26559	zinc finger, FYVE domain containing 27

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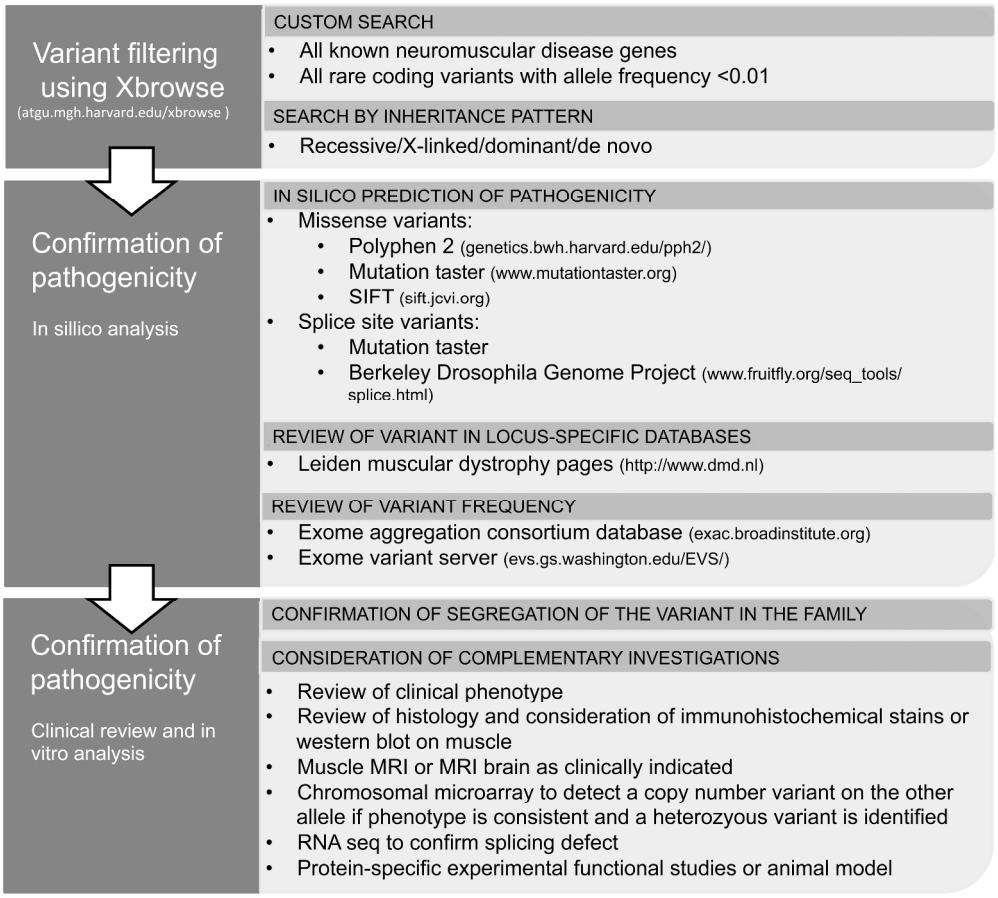

Whole exome sequencing analysis pipeline
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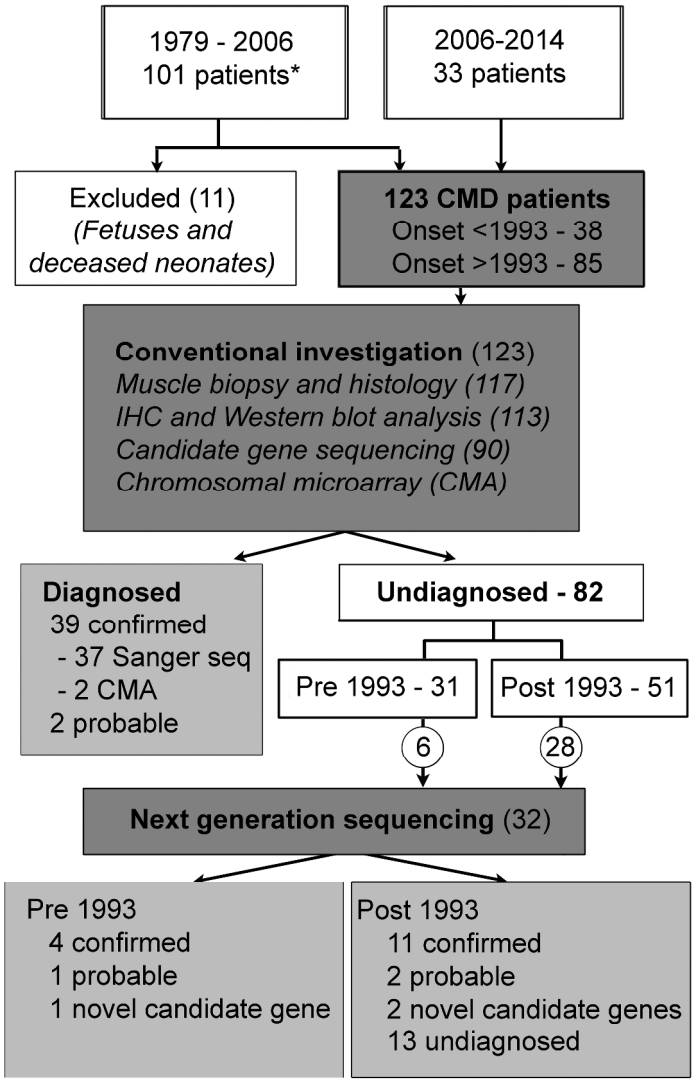

Cohort ascertainment and investigation

123 CMD patients were ascertained. 101 were part of a cohort published by Peat et al*. 11 fetuses and deceased neonates were excluded. Conventional investigation was with protein-based screening of muscle biopsy specimens, candidate gene sequencing and chromosomal microarray (CMA). Undiagnosed patients were investigated with Next Generation Sequencing (NGS) technologies, when available for consent. Follow up was more complete in patients presenting with onset of disease after 1993.
* These patients were included in the cohort published by Peat et al, Diagnosis and etiology of congenital muscular dystrophy, Neurology. 2008; 71:312-321

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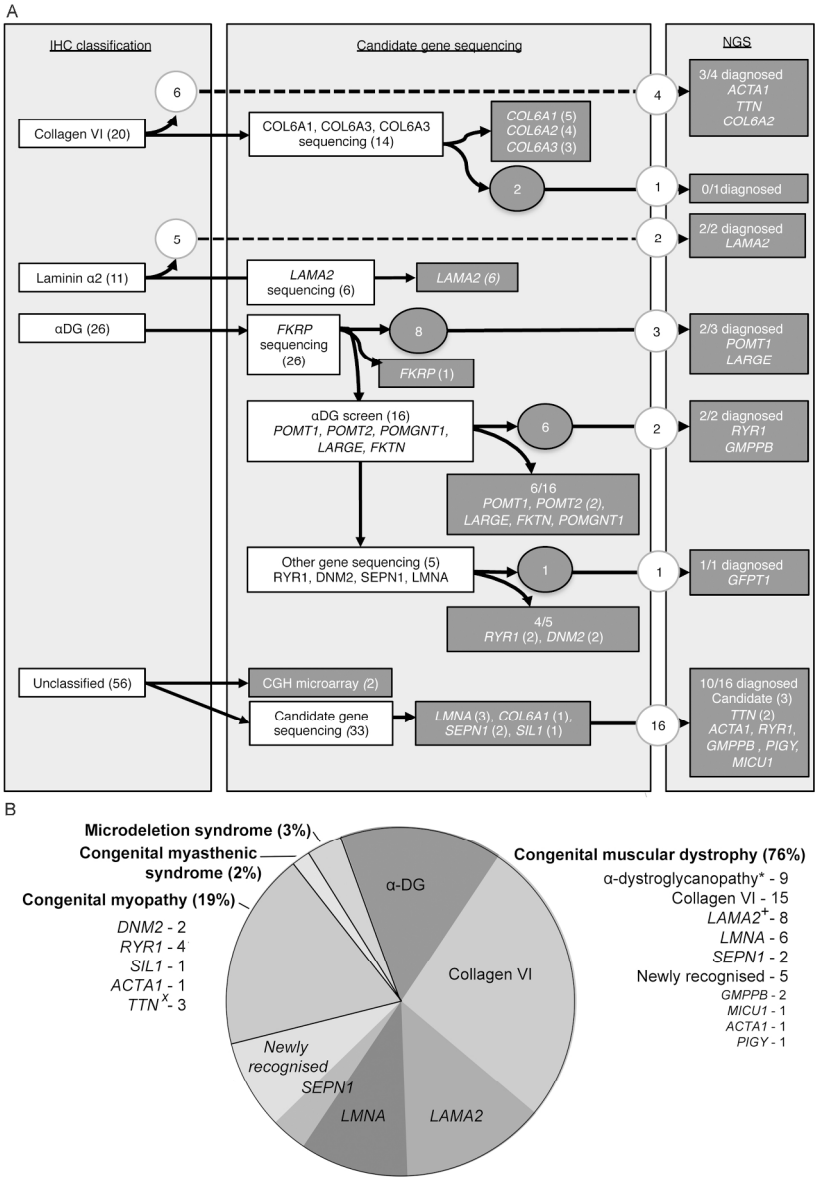

Diagnostic outcomes

A. Immunohistochemical analysis, candidate gene sequencing and genetic diagnoses:
Panel A illustrates the classification of patients by immunohistochemical (IHC) analysis and diagnoses made by candidate gene sequencing and next generation sequencing (NGS).
Left panel (IHC classification): 113 probands had immunohistochemical (IHC) analysis performed on muscle biopsy specimens. 57 probands were able to be classified on the basis of a moderate or severe reduction in collagen VI, laminin-α2 or glycosylated α-dystroglycan. 56 patients could not be classified by IHC analysis. Middle panel (Candidate gene sequencing): Candidate gene sequencing was performed on the basis of IHC classification, and when unclassified, on the basis of clinical phenotype. The gene sequenced is indicated in a white box, and the confirmed genetic diagnoses are shown by grey boxes. The number of patients undiagnosed after candidate gene sequencing is shown in a grey circle.
Right panel (NGS): NGS was performed on the number of patients indicated with a white circle. The

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confirmed diagnoses are shown in grey.

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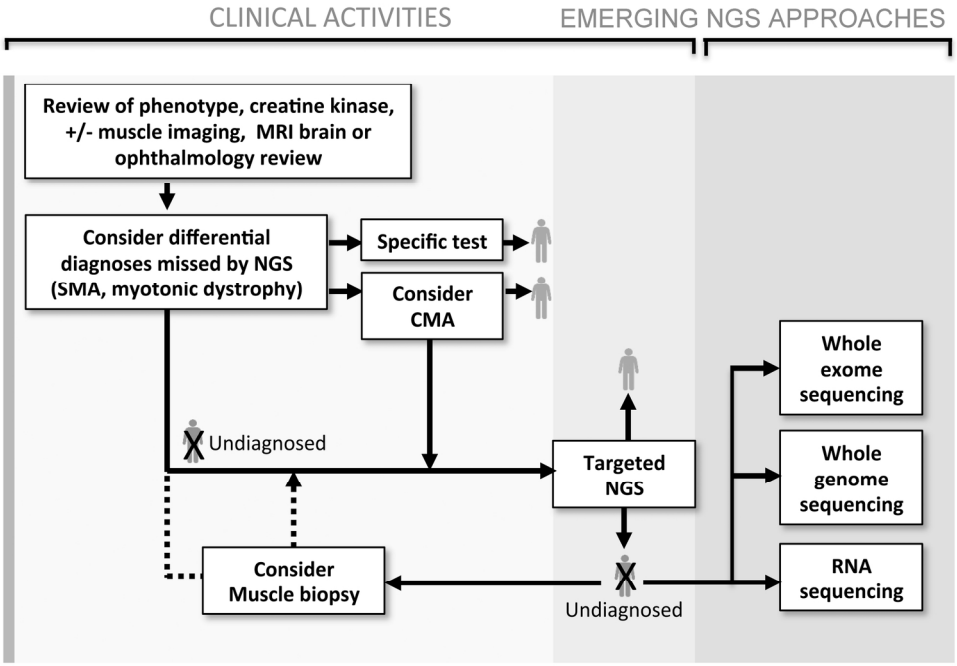

Proposed diagnostic algorithm for Congenital Muscular Dystrophy

Proposed diagnosis of suspected congenital muscular dystrophy patients using a targeted next generation sequencing neuromuscular gene panel after exclusion of diagnoses missed by this technology. Muscle biopsy should be considered in patients undiagnosed by NGS.
CMA, chromosomal microarray; SMA, spinal muscular atrophy; NGS, next generation sequencing; RNA, RNA sequencing

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