Delineation of the genotype and phenotype of children presenting with dystrophies, excluding dystrophinophathies, in the Western Cape of South Africa. (2019-2020)

Sub-study of the ethics approved Database / Registry

Inherited Neuromuscular Disease research: moving towards a definitive molecular diagnosis for each patient Ref Rec R030/2018

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Declaration / plagarism statement

DECLARATION

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ABSTRACT

Background

Muscular dystrophies (MD) and myopathies are a distinct group of clinically and genetically heterogeneous inherited muscle diseases. They cause muscle weakness often with cardiac, pulmonary, and musculoskeletal dysfunction, leading to reduced longevity. MDs and myopathies present across all life stages. Delineation of this condition and specifically the subgroups with additional connective tissue involvement is poorly described in sub-Saharan African populations.

Aim

To delineate the phenotypic, and where possible genotypic expression, of muscular dystrophies and myopathies with connective tissue involvement in an African setting.

Methods

A retrospective cohort study was undertaken of children with muscular dystrophy / myopathy and connective tissue involvement who attend a dedicated neuromuscular service. Patient demographics, diagnosis and clinical profile was collated. Patients were allocated into two groups, congenial /infantile and childhood, based on age of onset. Muscle biopsy characteristics, biochemical findings, and where available, genetic analysis were captured. Based on the combined findings children were categorised into connective tissue variant groups i.e., Collagen 6 related myopathies, Rigid Spine Syndrome (*SELENON* phenotype), *LMNA*-related, *ACTA1* related myopathies, MDC1A, and a subgroup who could not be categorised. Descriptive statistics and categorical variables were compared to evaluate primary study questions. Ethical approval was obtained by the University of Cape Town Human Research Ethics Committee (HREC:549/2019). Families gave informed consent prior to enrolment.

Results

A total of 57 children were reviewed, 50 of whom met the inclusion criteria of connective tissue spectrum in the setting of muscle disease (female to male ratio 1.3:1). There was a predominance in children from African ancestries, followed by those of European descent. 31/50 (62%) presented in the congenital-infantile age period, the remainder presented after 2 years of age. Children with congenital/infantile onset were more likely to lose independent ambulation compared to children with childhood onset (5/8, 62% vs 3/8 38%). Scoliosis

complicated the course in 29/50 (58%) children, again affecting congenital/infantile onset children more when compared to the childhood onset group (19/29, 65%, vs 10/29,35%); (p=0.003), and spinal rigidity was more prevalent in the congenital/infantile onset compared to the childhood group, (8/11 (73%) vs 3/11 (27%)). The childhood group were statistically more at risk of suffering compromising respiratory muscle dysfunction (32/45, 71%, vs 13/45,29%) ;(p=0.04). Genetic diagnosis was available for 9 patients. Based on this and the combined phenotypes n=17 were considered part of the Collagen 6 group, n=7 Rigid Spine Syndrome and n=14 LMNA spectrum, n=3 under the *ACTA1* mutation expression, n=2 LAMA2 and the remaining 7 could not be categorized or did not fall under one of the main groupings.

Conclusion

This study confirmed expression of this subgroup of muscular diseases with connective tissue involvement within the SSA population. The burden of disease from these conditions is across multiple systems and significant, requiring specialized care. Early recognition and referral to neuromuscular centre, would improve the potential outcomes for these children with collaborative multidisciplinary team. Serum creatine kinase (CK) levels and clinical markers such as rigid spine, dropped head, and skin laxity could be used in resource limited settings for probable and possible phenotype.

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LIST OF ABBREVIATIONS

MD	Muscular dystrophies
CMs	Congenital myopathies
CMD	Congenital Muscular Dystrophy
SMA	Spinal Muscular Atrophy
HREC	Human research and Ethics Committee
RCWMCH	Red Cross War Memorial Children's Hospital
UCT	University of Cape Town
NM	Nemaline Myopathies
NNIV	Nocturnal Non-Invasive Ventilation
EDMD	Emery-Dreifuss Muscular Dystrophy
VUS	Variant of Uncertain Significance
RSS	Rigid Spine Syndrome
MEB	Muscle-Eye-Brain Disease
COL6A	Collagen Type 6 A
WWS	Walker-Warburg Syndrome
MDCIA	Congenital Muscular Dystrophy Type 1A
LAMA2-MD	laminin alpha 2 muscular dystrophy

SELENON	Previously called also called SEPN1 or selenoprotein N
CMDmt	Congenital Muscular Dystrophy with Mitochondrial Structural Abnormalities
LMNA	lamin A Gene
LMNA-RD	LMNA-Related Dystrophy
ACTA1	Actin Alpha 1
LGMD	Limb-Girdle Muscular Dystrophy
CK	Creatine Kinase
T2W MRI	T2 Weighted Magnetic Resonance Imaging
CFTD	Congenital Fibre Type Disproportion
RSMD	Rigid Spine with Muscular Dystrophy
CMT	Charcot-Marie Tooth Disease
AIDP	Acquired Inflammatory Demyelinating Polyradiculoneuropathy
COX	Cytochrome C Oxidase
NADH	Nicotinamide Adenine Dinucleotide + Hydrogen
RYR 1	Ryanodine Receptor 1

Glossary of terms

Muscular dystrophies (MD) : Muscular dystrophies (MD) and myopathies are a distinct group of clinically and genetically heterogeneous inherited muscle diseases. They cause muscle weakness often with cardiac, pulmonary, and musculoskeletal dysfunction, leading to reduced longevity.

Myopathies: Myopathies: a distinguished group of inherited muscle diseases, which are clinically and genetically different. The most frequent clinical presentation is in the neonatal period as a floppy infant; however, congenital myopathies (CM) can present at virtually all life stages, making them an essential diagnostic consideration in all individuals with muscle dysfunction.

Muscular dystrophies (MD): with connective tissue variant are a collection of genetically, clinically, and biochemically different entities sharing clinical characteristics defined by connective tissue involvement.

Genotype: The consequent mixture of alleles that a person controls for a specific gene, a person inherits a couple of same alleles, their genotype is deemed to be homozygous at that locus, if they hold two distinct alleles, their genotype is classified as heterozygous for that locus.

Phenotype: The observable characteristics or traits of an individual that are produced by the interaction of the genotype and the environment **(** the physical expression of one or more genes).

Gene mutation: is a constant change in the DNA chain that makes up a gene, such that the order varies from what is observed in most people. Mutations can point to differences in the structure of an encoded protein or a reduction or total failure in its character because a shift in the DNA sequence hits all copies of the encoded protein.

Pathogenic variant: A genetic modification raises an individual's choice for a certain disorder. If such a modification (or mutation) is received, the gain of symptoms is more possible, but not guaranteed. Also called a harmful mutation, disease-causing mutation, predisposing mutation, and susceptibility gene mutation.

A variant of uncertain (or unknown) significance (VUS): is a genetic modification that has been recognised by genetic examination but whose importance to the role is not known.

Rigid spine syndrome is described by a marked defect in the flexion of the spine and gradual development of scoliosis leading to reduced respiratory vital capacity and respiratory failure. Joints contractures have commonly been described.

CHAPTER 1 INTRODUCTION AND LITERATURE REVIEW

1.1 Background

Muscular dystrophies (MD) and myopathies are a distinct group of clinically and genetically heterogeneous inherited muscle diseases. They cause muscle weakness often with cardiac, pulmonary, and musculoskeletal dysfunction, leading to reduced longevity. MDs present across all life stages, making them an important diagnostic consideration in all individuals with muscle weakness (1). Muscular dystrophies cause progressive muscle wasting (damage) and weakness, especially affecting limb muscles. In some cases, it may affect the muscles of the face and muscles that assist breathing and swallowing (2).

The prevalence of congenital muscular dystrophies (CMD) is incompletely defined. A systematic review in 2016 reported an estimated overall prevalence of 0.99 per 100,000, but data from Africa was lacking(3, 4). Delineation of the genetics of the CMDs are also relatively limited. This is especially the case in Africa, compounded but the lack of expertise and access to high throughput technologies which limits discovery of novel candidate variants which are likely to exist based on the genetic heterogeneity of African populations (3, 4). Another meta-analysis reported the overall pooled prevalence of combined muscular dystrophies was 16.14 (confidence interval [CI],11.21-23.23) per 100,000.(5). These estimates are likely to underestimate the true prevalence based on the increasing recognition of the condition and the delineation of an expanded range of clinical phenotypes under the congenital myopathy umbrella(1). The natural history of particular subgroups is reported in only a few cohorts, not all of which were fully genetically delineated (6).

Most MDs are of autosomal recessive inheritance, but dominant mutations occur in the setting of de novo mutations e.g., Ullrich CMD (7). MDs are caused by mutations in genes responsible to produce proteins essential for contractile, cytoskeletal, signaling, or enzymatic function within the muscle fibres or extracellular matrix. The explosion in molecular genetics over the past three decades has brought a greater understanding of the biological basis for these disorders and the appreciation that different gene mutations, can cause similar phenotypes (8).

Conversely, mutations in one gene can cause very different phenotypes. Common themes to MDs are features of muscle fibre degeneration with inadequate cellular repair, leading to atrophy of these fibres and replacement by fat and connective tissue. This ends in the decline of function and joint contractures in skeletal muscle and some situations diminished cardiac and visceral (smooth muscle) capacity. In some muscular dystrophies scapular, humeral, and peroneal involvement with early onset of joint contractures and often cardiac involvement(8-10).

The management of muscular dystrophies has largely been symptomatic, with a strong focus on respiratory management, nutritional support, surgical correction of orthopaedic complications, and application of physical and occupational therapy services to maximize motor and respiratory function. For some congenital dystrophies, cardiac management is also necessary (11-13). Several candidate therapies are in preclinical study phases and due to enter the stage of clinical assessment (14). Evaluation of these drugs will hopefully lead to a new era of therapy for a group of previously untreatable and devastating diseases (15-17).

Each MD has distinctive features. Understanding the unique phenotype-genotype correlations has implications for genetic counselling inclusive of prenatal care, prognostic expectations and aspects of management as well as recruitment for clinical trials (18).

The current description of CMDs depends on the combined clinical features and cellular localization of the gene product and falls into five main categories.

I. C CMD due to mutations in genes encoding for proteins of the **basal lamina or extracellular matrix or receptors for extracellular matrix proteins**. This part includes mutations in the collagen 6 genes, laminin α2 (merosin), in integrin α7 and the more new modification due to integrin α9 gene insufficiency. Variations in DAG1 (dystroglycan 1 gene) have very lately also been reported.

II. Forms secondary to genes encoding for putative or confirmed glycosyltransferases that affect the **glycosylation of α-dystroglycan**. These include Fukuyama CMD, muscle-eye-brain (MEB) disease, Walker- Warburg syndrome (WWS), MDC1C and MDC1D, and other phenotypes associated with mutations in one of the 15 known genes.

III. Defects of **nuclear envelope proteins** (Lamin A/C gene (*LMNA)* and *nesprin*).

IV. Defects of proteins with thus far unknown function localized in the **endoplasmic reticulum**, which includes the form of CMD with rigid spine syndrome secondary to mutations in the *SEPN1* gene.

v. CMD with **mitochondrial structural abnormalities**(CMDmt) (19).

The term “congenital” refers to the pathological process commencing during the fetal period. This may not necessarily correlate with the timing of the clinical manifestation. As such it can be difficult to predict the onset of the clinical symptoms, affected children may develop symptoms at birth, but they may also start to exhibit weakness, contractures, and slowed motor milestones in the first year of life(19, 20) In some clinical spectra such as Ullrich CMD and SEPN1-related myopathies, (SELENON-related MDs), the onset can be delayed even beyond the end of the first year (21).

Muscular dystrophies (MD) with connective tissue variant are a group of genetically, clinically, and biochemically distinct entities sharing clinical features characterized by connective tissue involvement and include the following most well-delineated subtype:

I. **Collagen VI–related dystrophies** constitute a spectrum of severities from the mild Bethlem myopathy, intermediate severity subtype, through to the more severe Ullrich CMD. The disease is identified by a clinical combination of distal laxity, contractures of the proximal joints, skin changes, serum CK which is usually normal or only slightly raised. The most severe form, Ullrich CMD, is characterize by marked weakness concomitant with joint hyperlaxity and contractures at birth. Respiratory insufficiency worsens over time. Muscle biopsy can exhibit variable pathology of myopathic to clearly dystrophic involvement. Diagnosis is usually confirmed by identifying mutations in one of the three genes encoding one of the alpha chains of collagen type VI,*COL6A1*, *COL6A2* and *COL6A3*.

II. ***SELENON*-related MD**. SELENON-related MD. The clinical feature is identified by weakness and rigidity of the spine with early respiratory involvement. The onset of RSS in infancy is caused by mutations in the SELENON gene. Motor milestones are delayed but most children with SELENON -related CMD eventually achieve and maintain independent ambulation. Notwithstanding the spine rigidity, scoliosis typically emerges and may necessitate surgical intervention. Restrictive lung disease evolves later in the course usually in the early second decade while still ambulant. Cardiac involvement is not described. The creatine kinase level is typically normal and the muscle biopsy can have subtle dystrophic features or have multiminicores, fibre-type disproportion, or

myofibrillar changes with Mallory body-like inclusions. Scoliosis and respiratory insufficiency are almost inevitable findings in the second decade, with the probability of early signs from the first decade.

III. ***Lamin A/C*-associated CMD (*LMNA* gene)**.

IV. All patients present with weakness and gross motor delays in the first year of life, axial cervical muscle weakness, resulting in a “dropped head” phenotype—a distinct clinical feature of this syndrome. Progression of weakness is variable, but all patients have selective axial weakness and wasting of the cervic oaxial muscles. A rigid spine syndrome with thoracic lordosis evolves rapidly followed by the development of lower-limb flexion contractures at the knees and ankles. Cardiac dysfunction manifests with cardiac arrhythmias which may progress to cardiac failure. There is rapid progression of respiratory muscle involvement and early ventilatory support needed in the majority of cases. Creatine kinase levels are typically elevated. Muscle biopsy reveals myopathic and/or dystrophic features. Immunohistochemical studies are not helpful as a diagnostic marker. Hence DNA studies are needed for definitive diagnosis by identifying mutations in the LMNA gene. LMNA-related dystrophy (LMNA-RD) gene is associated with various phenotypes expressing skeletal muscle involvement: limb-girdle muscular dystrophy type 1B (LGMD1B), autosomal dominant Emery-Dreifuss muscular dystrophy (EDMD2); and the congenital muscular dystrophy (LMNA-CMD) described in this section(22). These clinical entities may express with the same LMNA mutation and coexist in the same family. The considerable clinical overlap supports that these phenotypes should be considered as a continuum in the clinical spectrum of LMNA-RD. The heart is affected in all three entities, with similar features, with an age of onset the main differentiating factor. The cardiac presentation may precede the onset of muscle weakness.

V. **MDC1A (LAMA) -related muscle disorders**.

Merosin-deficient CMD type 1A (MDC1A), caused by mutations in the *LAMA2* gene, constitutes approximately 50% of total CMD cases and is characterized by hypotonia within the first few months of life, increased levels of serum creatine kinase (CK), multiple joint contractures (23, 24). The *LAMA2* gene spans 65 exons and encodes the laminin-α2 chain, which assembles with laminin-β1 and -γ1 to form laminin-211 in skeletal muscles(25). The mutations within the *LAMA2* gene, without any identified hotspots, lead to complete or partial deficiency of laminin-α2. Patients with a partial

deficiency of laminin-α2 exhibit a milder phenotype, while a complete deficiency of the protein always results in severe phenotypes, such as white matter hyperintensities in the brain on T2W MRI (23). MDC1A is predominantly reported in European countries, with a smaller a number of cases described in Asia (26).

VI. ACTA1 related. myopathies (e.g. Nemaline myopathy, multiminicore and congenital fiber type disproportion).

Congenital myopathies are clinical and genetic different diseases identified by skeletal muscle weakness varying in severity. Three major forms have been recognised: actin myopathy, intranuclear rod myopathy, and nemaline myopathy. In SA CNM is the most common form. Internationally central core disease is the most common (27). Nemaline myopathies are a different group of congenital myopathies caused by de novo, dominantly or recessively inherited mutations in at least twelve genes. The genes encoding skeletal α-actin (ACTA1) and nebulin (NEB) are the most frequent genetic events (28), most patients have congenital onset distinguished by muscle weakness and hypotonia, but the spectrum of clinical phenotypes is wide, ranging from severe neonatal presentations to onset of a milder disease in childhood.(28, 29). Nemaline myopathy is a form of congenital myopathy described by the clinical phenotype that is extremely variable, with differing age at onset and severity. Muscle weakness typically involves proximal muscles, with the involvement of the facial, bulbar, and respiratory muscles. Nemaline myopathies are identified by the presence of structures that are rod-like or ovoid with electron microscopy, and with light microscopy stain red with the modified Gomori trichrome technique.(28, 29). Nemaline myopathy is further subdivided into seven groups according to severity, progressiveness, and age of onset. At the instant, five genes have been associated congenital myopathies. These include alpha-actin (ACTA1), alpha- and beta-tropomyosin (TPM3 and TPM2), troponin T (TNNT1), and nebulin (NEB) (32). he spectrum of clinical features of nemaline myopathies is wide, even in individuals with mutations in the corresponding gene, or in the same family. It ranges from neonates with severe disease and onset in utero, sometimes with fetal akinesia, to mild childhood-onset forms (33). Amongst patients with ACTA1 mutations, the severe form of nemaline myopathy with early-onset muscle weakness, rapid course, and respiratory insufficiency is most frequently reported. A benign but rare phenotype is reported (34). Cardiomyopathies have not typically reported in ACTA1 myopathies (35). Multi-minicore disease is a recessively inherited neuromuscular disorder characterized

by multiple cores on muscle biopsy and clinical features of congenital myopathy. There is marked clinical variability which corresponds to genetic heterogeneity (36).

Mutations in the skeletal muscle actin gene, ACTA1 are responsible for up variations in the skeletal muscle actin gene, ACTA1 are accountable for up to 20% of congenital myopathies with a kind of pathologies that covers nemaline myopathy, intranuclear rod myopathy, actin myopathy and congenital fibre type disproportion(37).

Congenital fibre type disproportion (CFTD) is a disease of skeletal muscle with weakness and hypotonia started at birth or shortly thereafter, the generally quiet or absent progression of motor symptoms and frequent skeletal abnormalities, including congenital hip dislocation, joint contractures, foot deformities and kyphoscoliosis. Mutations in the α-skeletal actin (ACTA1) gene have been identified recently in severe cases of CFTD, but the molecular mechanisms leading to disproportion in fibre size are unknown Whether ACTA1 mutations can also lead to milder cases of CFTD needs to be investigated(39). Congenital fibre type disproportion (CFTD) is viewed as a non-progressive or gradually progressive muscle disease with the relative smallness of type 1 fibres on pathological examination. Positive family history has been reported in about 40% of cases, but the inheritance pattern is not clear. Both autosomal recessive and dominant modes of inheritance have been suggested (40).

VII. Miscellaneous (integrin and nesprin groups, and the non-specific or undefined phenotypes)

Nesprin 1, is a part linker of nucleoskeleton and cytoskeleton (LINC) complex and the cytoskeleton and errors in nuclear positioning and abnormal aggregation of nuclei happen in many muscle disorders and correlate with muscle dysfunction, nesprin 1, which includes multiple isoforms, is an integral element of the complex, critical for nuclear positioning and anchorage in skeletal muscle, and is considered to implement a fundamental link between nuclei and actin(41, 42). Integrins are transmembrane heterodimers of two different subunits, α and β, associated by non-covalent interactions. In humans, at least 18 different α and 8 different β subunits are known, resulting in 22 distinct heterodimers 1,2 The α7 subunit is mainly displayed in skeletal and cardiac muscle, while the β1 chain is expressed completely in the body and linked to other various α subunits. Integrin α7 in skeletal muscle localizes at the sarcolemma, at the neuromuscular terminals, and, prominently at the myotendinous junctions where it gives an anchorage for laminin α2, conferring mechanical stability and traction resistance to the skeletal muscle fibre(43).

1.2 Motivation/Purpose of the study:

Muscle diseases, in-line with other hereditary and non-communicable diseases, are under-recognized and under-researched in the African setting where health care needs are dominated by communicable disorders. As such the true burden of disease from neuromuscular disorders in Africa is not known, especially for rare sub-types of muscular dystrophies and myopathies.

1.3 Study goal

To delineate the phenotypic, and where possible genotypic expression, of muscular dystrophies / myopathies with connective tissue variants in an African setting.

1.4 Research questions:

What types of muscular dystrophies / myopathies with connective tissue variants are managed in a dedicated neuromuscular service in sub-Saharan Africa? How do their clinical characteristics, histological findings, genetic profile, management, morbidities, and outcomes differ from internationally reported populations? Can specific clinical markers assist diagnostic direction in the early detection and delineation of this group?

1.5 Study Objectives

I. To describe the features of children diagnosed with muscular dystrophies / myopathies with connective tissue variants presenting to the neuromuscular service at the Red Cross War Memorial Children's Hospital (RCWMCH).
II. To review the histological and where available the genetic profile, and to correlate this with disease subtypes and severity.
III. To understand the burden of disease suffered by children with this disease spectrum.
IV. To develop early recognition tools to encourage timeous referral to specialised units for intervention.
V. To identify effective management pathways in the local setting.

VI. To compare the range of disease expression in the South African setting compared to the internationally reported cohorts.

VII. To delineate in detail this sub-group with connective tissue MD variant.

1.6 LITERATURE REVIEW

1.6.1 Introduction

This section examines the literature on the subject to provide a summary of the existing knowledge of the clinical and genetic phenotype of muscular dystrophies and myopathies associated with connective tissue spectrum. A systematic literature search was undertaken. This focused on reports of muscular dystrophies and myopathies with connective tissue spectrum, especially for the phenotype, clinical course and gene expressions published between 01/01/1960 and 30/10/2020. Hand searches of reference lists of recognised articles were also conducted. Search terms included 'muscular dystrophy' OR 'myopathy' AND 'connective tissue spectrum' OR 'connective tissue variant' OR 'ligament laxity' OR 'joint laxity' (PubMed, MEDLINE), inclusive of MESH terms where available. Further searches of reports of clinical phenotype expression were performed under each of the key connective tissue variant subtypes ie "Collagen 6 related myopathies", "Rigid Spine Syndrome", "SELENON-related MD", "Lamin A/C-associated CMD (LMNA gene), MDC1A (LAMA) -related muscle disorders", ACTA1 related myopathies, Congenital Fibre Type Disproportion and Multiminicore myopathy.(19, 20). Human clinical studies were included with case reports and series permitted due to the rarity of some of the subtypes. English language reports were selected. Review articles were assessed to capture reports otherwise missed.

Studies were excluded if they were published prior to 1960 as formal descriptions of many neuromuscular disorders were not established until the late 1950s and it would be difficult to perform comparisons between earlier diagnoses and modern diagnostic descriptions. Studies reporting a founder effect were therefore also excluded from the review. Titles and abstracts for all citations were evaluated for potential inclusion in the review. Full articles were obtained for studies meeting the inclusion criteria where possible. Duplicate papers reporting on the corresponding data were excluded.

Search term, Muscular Dystrophy, and connective tissue spectrum this resulted in articles (n= 18), muscular dystrophy and ligament laxity OR joint laxity, (n= 603), myopathy and connective tissue (n = 34), myopathy and ligament laxity or joint laxity (n = 59), Collagen 6 related myopathies (n =112), Rigid Spine Syndrome (n =151), laminin related myopathy (n= 218), LAMA 2 -related muscle disorders,(n=28), SELENON - related myopathy (n= 29), ACTA1 - related myopathies (n = 57). Total studies (n=1338), after duplications were removed (n =654), after abstract / full report reviewed (n= 684). Remaining studies were a total (n= 51) to be reviewed, with Collagen VI–related dystrophies (n=12), SELENON-related CMD (n =11), Lamin Gene associated CMD (LMNA gene) (n= 7), ACTA1 related myopathies(n = 18), and miscellaneous (n = 3).

Collagen VI-related muscle disorders: (n=112) articles, 12 covered the study question, all were Observational studies.

Norwood et al found the prevalence of the collagen VI-related muscle disorders was 0.13 cases per 100,000 of the population in northern England (44). A Japanese study reported prevalence to be 0.77 cases per 100,000 and collagen VI deficiency is the second most common CMD after Fukuyama type CMD in Japan (45). Table 1 summaries the major features of these papers. Overall children with collagen VI-related muscle disorders had striking hyperlaxity, especially of the distal joints. Age of onset ranges from congenital, with reports of fetal hypokinesia, to slowly progressive proximal muscle weakness and contracture in late childhood (46-48). Ullrich Congenital Muscular dystrophy has a mean onset of symptoms of 12 months of age. it is characterized by severe clinical course with neonatal muscle weakness, proximal joint contractures, hyperlaxity of the distal joints, failure to thrive, and in the majority lack of independent ambulation, and severe respiratory impairment by the end of the first decade of life (46, 47) (49).Transient feeding problems might also occur in the early neonatal period (50). Contracture development is common with slowly progressive deterioration in motor function(50-52). Ambulation can be achieved but potentially lost in later life(50). Bethlem myopathy is clinically heterogeneous and in general the clinical course is thought to be benign. Jobsis et al.(47), longitudinally followed 23 children and 36 adults, and found that nearly all children exhibited weakness or contractures during the first two years of life. The group emphasized that Bethlem myopathy can be slowly progressive and culminate in wheelchair use (47).Respiratory involvement is rare in congenital-infantile onset but common in late-onset with severe respiratory involvement requiring ventilatory support in the first or second decade of life (46, 50). This is

further exacerbated by development of scoliosis. The condition can also manifest with skin laxity and tendency to abnormal scar formation after injury (53). Tetreault et al reported a group of French-Canadian patients, from 11 families, suffering from a CMD with joint hyperlaxity (CMDH) with clinical overlap with Ullrich CMD. They share with Ullrich CMD the presence of congenital hypotonia, weakness, contractures, distal joint hyperlaxity, scoliosis, normal intelligence, and constantly delayed motor milestones. But the clinical features were less severe than in Ullrich CMD: they obtained independent walking and do not manifest respiratory failure notwithstanding scoliosis (54). Management is mainly symptomatic, with physiotherapy, and appropriate respiratory care is reported to improve, potentially prevent, or delay, ventilation related complications. Timely scoliosis management is paramount (50).

The CK level is typically normal or only mildly elevated(6, 46, 47). Early muscle histological changes in patients with Ullrich collagen muscular dystrophy include dystrophic features (muscle fibres degeneration, regeneration, and inflammation) as the main finding (55). Immunohistochemical examination reveals a complete absence of collagen VI in the muscle in most but not all biopsies, as normally collagen staining can occur (56). The biopsies from the French-Canadian cohort showed variation in fibre size, central nuclei and increased endomysial connective tissue (54).There are three major collagen VI genes, COL6A1, COL6A2, and COL6A3. COL6A1 and COL6A2 lie on chromosome 21, whereas COL6A3 is on chromosome 2(48). Collagen VI mutations associated with the Ullrich CMD, or Bethlem myopathy spectra of disorders are associated with mutations in the COL6A1, COL6A2, and COL6A3 genes(57, 58). The other mutations, COL6A5 and COL6A6, are not associated with CMD, likewise COL29A1 and COL6A5 which is linked to atopic dermatitis (57, 58).

SELENON-related myopathy:(n=29) articles, 11 covered the study question, all were Observational studies

Rigid spine syndrome (RSS) in the majority of cases is associated with generalized muscular weakness, prominent neck weakness in infancy, early onset spinal rigidity, and respiratory insufficiency (59-63). Onset of symptoms range from birth to early infancy, with reduced fetal movements, and transient feeding difficulties also reported(62, 64). Even presumed juvenile onset cases have evidence of involvement from infancy(63, 65, 66). Ophthalmoparesis is also reported in severe cases(63). Mild progression in motor function occurs with loss of ambulation reported in some children, as well as progressive scoliosis occur in early childhood period (63). Restrictive respiratory pattern is typical, with central

apnoea reported, requiring non-invasive nocturnal ventilatory support in some patients in the late childhood period(60, 63, 65-67). Retained ambulation at the time that respiratory dysfunction manifests can occur such that there is disparity between muscle dysfunction and respiratory weakness (67). Long-term nocturnal non-invasive ventilation can lead to significant improvement in function and quality of life. Cardiac arrhythmias are reported but tend to be asymptomatic (60). Jorgensen, *et al* reported an existence of malignant hyperthermia in a patient with rigid spine syndrome a perioperative complication in a 14-year-old with rigid spine phenotype. The serum creatine kinase levels are normal to mildly raised (68). Muscle histopathology shows insignificant myopathic changes to severe fibre degeneration and absent selenoprotein immunostaining (61, 64). Severe dystrophic changes are also reported (61, 65). Multi-minicores were the most common lesion (59.5%) in one case series of RSS patients, often also associated with mild dystrophic features and occasionally with eosinophilic inclusions (63).

Schara et al reported no correlation between the type of mutation and the severity of the phenotype. Clinically, the children present as floppy infants with a marked delay of motor and mental development. The onset was within the first two years of life with muscle weakness. The gross motor development, although with delay, was achieved in most of the patients, who maintain ambulation for a long period. The rigid spine was observed at a mean age of 10 years. All patients develop respiratory impairment and mark muscular atrophy. Intermittently nocturnally ventilation may be necessary for teenage(65).

SELENON1 may perform a pivotal role in the biology of skeletal muscles such as the diaphragm, by controlling the redox environment of the cells and preventing it from oxidant injury(59). Despite the physiological functions of the molecule being well established, the exact pathophysiology remains unclear, and to date there is no targeted treatment (69, 70).

LMNA - related myopathy: (n=218) articles ,7 covered the study question, all were Observational studies

The term Laminopathies are generally categorised into four clinical groups, namely Emery–Dreifuss, LMNA-related congenital muscular dystrophies, limb-girdle muscular dystrophy type 1B (LGMD-1B) and dilated cardiomyopathy with conduction defects with no clear genotype-phenotype relationships(71). Mutations in the *LMNA* gene occur in children with severe axial weakness and wasting of cervical axial muscles, referred to as the “dropped head” syndrome phenotype, often accompanied by feeding and respiratory difficulties.(72-74). Patients typically present with selective axial weakness and wasting of the cervical

axial muscles. Limb involvement is predominantly proximal in upper limbs and distal in lower limbs. Feet deformities and a rigid spine with thoracic lordosis manifest early during the clinical course. Contractures developed later, most frequently occurred in the extremities. Non-invasive ventilatory support is commonly needed. Cardiac arrhythmias also occur in some 40% at an older age but are asymptomatic in the majority of cases. The LMNA gene is associated with a broad variety of clinical spectra in the same family (70). Creatine kinase levels were mild to moderately increased (74).

Muscle biopsies have dystrophic changes in 60% of cases and nonspecific myopathic changes are also reported. Markedly atrophic fibres were common, most often type 1, and a few patients show inflammatory changes. However the changes are not specific enough to be diagnostic of a LMNA-related myopathy. As such the only definite way to diagnose this condition is with molecular techniques like *LMNA* gene sequencing (75). LMNA encodes for Lamin A/C, type V intermediate filaments that polymerise beneath the inner nuclear membrane to form the nuclear lamina. Lamin A/C involves roles in nuclear stability to mechanical strain and gene control(76, 77). Mutations in the *LMNA* gene should be sought in any infant with dystrophic features and normal immunohistochemical examination; dominant axial, proximal muscle weakness, spine rigidity, and joint contractures(78) . The most frequent diseases associated with mutations in the LMNA gene are characterized by skeletal and cardiac muscle involvement, scoliosis frequent in all forms of the disease and spinal rigidity is noted in LMNA-CMD (Congenital infantile form, CK normal to mildly raised, Non-autonomous ambulation frequent in LMNA-CMD, muscle biopsy showed unspecific myopathy or dystrophies(22).

LAMA2 - related muscle disorders: (n=28) articles, 7 covered the study question, all Observational studies**.**

MDC1A is usually produced by complete merosin deficiency resulting in critical muscle weakness with onset within the first 6 months of life, contractures, respiratory insufficiency, failure to complete independent ambulation, and elevated creatinine kinase (CK). Less common clinical characteristics include seizures and intellectual disability (14). In the less severe forms of the disease present later in infancy or childhood with delayed gross motor milestones, hypotonia, and elevated CK. They have slowly progressive weakness that simulates limb-girdle muscular dystrophy. Generally the clinical phenotype depends, in part, on the amount of laminin α2 present (79, 80). In two reports Geranmayeh et al and Cotta et al, they found complete laminin-2 loss results in an early-onset, a congenital form

characterized by severe hypotonia, muscle weakness, contractures, non-ambulation, and respiratory insufficiency, and partial loss of laminin-α2 presented as a late-onset, limb girdle-type muscular dystrophy(79, 80). The clinical categories of MDC1A with a complete absence of laminin alpha-2 chain expression usually match with a severe phenotype characterised by muscle weakness and atrophy, diffuse joint contractures, failure to achieve independent ambulation, raised creatine kinase (CK) level and unique white matter hypodensities on cranial magnetic resonance imaging (MRI). Cardiac involvement has been reported in cases with late-onset LAMA2-MD. There are only a few reports of cardiac involvement in individuals with MDC1A; however, as improved medical care prolongs life expectancy, cardiac involvement may become more prevalent, and thus a management concern (81, 82).

Oliveira et al in their comprehensive study described detailed clinal phenotypes: The clinical spectra of LAMA2 muscular dystrophy include severe congenital muscular dystrophy type 1A (MDC1A) to milder late-onset LAMA2-MD. MDC1A is typically identified by neonatal hypotonia, reduced spontaneous movements, and respiratory insufficiency. Failure to grow, gastroesophageal reflux, aspiration, and repeated chest infections requiring numerous hospitalizations are common. Late-onset LAMA2-MD manifests range from early childhood to adulthood (84). Affected individuals may have muscle hypertrophy and develop a rigid spine with joint contractures, scoliosis, and cardiomyopathy can occur late childhood period. Respiratory involvement is caused by progressively restrictive chest wall defects. Recurrent Chest infections may cause atelectasis, which along with limited pulmonary reserve, increases the risk of acute respiratory failure in the setting of infection (83). Limitation of eye movements (ophthalmoparesis) may be evident as early as age two years (83). The requirement for ventilatory assistance is most likely to happen in two time periods between, birth and age five years in the most severely affected infants mainly due to respiratory muscle weakness, ventilatory support may be non-invasive or mechanical with a tracheostomy (80), and between 10 - 15 years due to progressive restrictive lung disease-causing to respiratory insufficiency (84). The common biopsy findings was dystrophic changes(71, 84), however. Scoliosis is frequently observed from the first decade of life, it is often slowly progressive and may contribute to respiratory insufficiency (85).

In classic MDC1A phenotype, molecular analysis is not necessary, the final diagnosis is made by muscular biopsy, muscle biopsy confirmed perimysial fibrosis as well as variation in fibre size and necrosis supported by the elevated CK level (86). The CK level in

MDC1A through the first years of life is slightly elevated (83, 87), in the Late-onset LAMA2-MD usually high during the clinical course (83).

ACTA1 related myopathies (multiminicore, NEM, CFTD) : (n=18) articles, 6 covered the study question, all Observational studies

Mutations in the skeletal muscle actin gene, ACTA1 are responsible for up variations in the skeletal muscle actin gene, ACTA1 are accountable for up to 20% of congenital myopathies with a kind of pathologies that covers nemaline myopathy, intranuclear rod myopathy, actin myopathy and congenital fibre type disproportion(37).

Ilkovski, et al described a few cases with *ACTA1* mutations that show a cardiac dysfunction including cardiomyopathies, despite skeletal muscle α-actin accounting for around 20% of the striated muscle α-actin in the heart (88). In their cohort Colombo et al described that *ACTA1* of subgroup congenital myopathy required nocturnal non-invasive ventilation and gastro Jejunostomy in a high percentage, highlighting that, except early lethal *ACTA1* cases, bulbar and respiratory complications are comparable over time, NNIV was started in patients with ACTA1 mutation at a mean age of 8.5 years, the majority of patients with the ACTA1 mutation achieving independent ambulation. Antenatal onset was evidence by frequently reduced fetal movements in 37% and/or polyhydramnios (23%) The prematurity rate was increased. Neonatal bulbar and respiratory involvement requiring tube feeding (25%) and ventilatory support were common in *ACTA 1* mutation (33). The most common clinical presentation of *ACTA1* diseases is severe congenital myopathy phenotype, of global hypotonia, distal weakness and at birth likely no spontaneous movements, myopathic facies, high arched palate, no spontaneous respiration or poor respiratory function, and a poor suck, death frequently results before 1 year of age (89). Nevertheless, *ACTA1* diseases have a range of severity. Some of the even more severely affected subjects are diagnosed with foetal akinesia and thus have onset early in utero, whilst others have late-onset (90). Ferreiro et al, in their research of case series clinical and histologic Findings in ACTA1-related nemaline myopathy reported a variety of clinical spectrum consisting of generalized muscle weakness that affects predominantly the pelvic girdle, and includes hand weakness, amyotrophy, spinal rigidity and hyperlaxity; scoliosis and respiratory involvement were unusual findings, they conclude their study as despite the

recognized phenotypic variability in ACTA1-related nemaline myopathy, clinical and histological presentations appear to correlate with the position of the mutation, which confirms emerging genotype/phenotype correlations and better predicts the prognosis of the affected patient (91).

Table 1. Literature review, articles were selected based on those most relevant to study question which provided detailed phenotypic and where possible genotypic descriptions of the typical sub-types.

Study/Authors/Title	Study subtype	Country/ Location	Year	Findings of the study
Lampe et al. (46)	Collagen VI-related muscle disorders.	USA	1993	Observational study Clinical findings : Fetal hypokinesia, neonatal hypotonia, weakness, feeding problems and some cases respiratory distress, striking hyperlaxity, especially of the distal joints. Diagnosis depends on typical clinical features and muscle biopsy characteristics. Outcome: The clinical course was usually slowly progression. Outcome: Respiratory involvement ,Ulrich form often results in later loss of ambulation and severe respiratory involvement requiring ventilatory support in the first or second decade of life
Jobsis et al. (47)	Collagen VI-related muscle disorders.	Europe, Netherlands	1999	Clinical findings (n=7): Clinical observational study, early-onset diminished fetal movements, hypotonia, contractures, striking hyperlaxity of distal joints. Diagnosis depends on phenotype and genetics. Outcome: During childhood muscle weakness remained stable but the deterioration of walking noted. .
Nadeau et al. (50)	Collagen VI-related muscle disorders.	Europe/ UK	2009	Clinical findings and course (n=13), clinical retrospective observational study, the mean age at onset of symptoms was 12 months. Transient feeding problems might occur in the early neonatal period. (61.5%) achieved independent ambulation at a mean age of 1.7 years. (69.2%) became wheelchair dependent at a mean age of 11.1 years.(70%) nocturnal non-invasive ventilation commenced in the mid adolescent period. Outcome: Further deterioration occurred with development of scoliosis in the first decade of life. All cases were diagnosed based on.

Bonnemann etal. (49)	Collagen VI-related muscle disorders.	USA	2010	Clinical findings and course: Observational study. Early-onset weakness linked with obvious distal joint hyperlaxity and the early onset or early progression of more proximal contractures. In the most severe cases ambulation is not achieved. In Bethlem myopathy characterized by of early or later onset, but is milder in its manifestations, typically allowing for ambulation well into adulthood, whereas typical joint contractures are frequently prominent.
Schessl J etal. (55)	Collagen VI-related muscle disorders.	USA	2008	Muscle biopsies were analyzed from (n= 8) UCMD patients ranging in age from 6 to 30 month. Early histological changes in the muscle of patients with Ullrich collagen muscular dystrophy include dystrophic features (muscle fibres degeneration, regeneration, and inflammation) as the predominant finding.
Moghadaszadeh B etal. (59)	SELENON -related muscular dystrophy	USA	2001	Clinical findings and course: SEPN1-related myopathy (rigid spine syndrome (RSS)) in the majority of cases is associated with generalized muscular weakness, late-onset spinal rigidity, and respiratory insufficiency, most commence non-invasive ventilation in the late childhood period. These patients have normal levels of serum creatine kinase.
Villar-Quile etal. (63)	SELENON -related muscular dystrophy	Europe ,France	2013	large international case series. Clinical findings and course (n=132), severe axial muscle weakness, spinal rigidity, and scoliosis, with relatively conserved limb strength and ophthalmoparesis in severe cases. All patients developed respiratory failure (from 10.1+/-6 years), 81.7% requiring ventilation while ambulant. Outcome: Reduced the functional capacity, non-invasive nocturnal ventilatory support.
Koul etal. (64)	SELENON -related muscular dystrophy	Oman, Middle East	2015	Clinical findings and course: retrospective observational study (n=12).diagnosis made on clinical and biopsy, Onset of the disease ranged from birth to 18 months of age. , reduced fetal movements, and transient feeding difficulties reported in three patients. Outcome: progression of scoliosis and of contractures reduced the functional capacity in six patients. A restrictive ventilatory defect.
Flanigan KM etal.	SELENON -related	USA	2001	Clinical findings and course: (n=4). Case series, all patients had hypotonia and prominent neck

	muscular dystrophy			weakness in infancy, with the development of spinal rigidity, and early scoliosis in late childhood/adolescence. Muscle biopsy showed minimal, nonspecific myopathic changes. Clinical course: Slowly progressive weakness and early respiratory insufficiency requiring non-invasive ventilation.
Silwal et al.(62)	SELENON -related muscular dystrophy	Europe/ UK	2020	Clinical findings and course: (n=60): Cross-sectional multicentre study, diagnosis made on clinical phenotype and genetics review. The majority of patients (77%) had onset of symptoms in the congenial-infantile range ,early feeding difficulties, hypotonia, poor head/neck control, and developmental delay as common presentations, 60% of the patients were non-ambulant. 83% had progressive respiratory insufficiency, requiring nocturnal non-invasive ventilatory support. Clinical course: A severe reduction in Forced Vital Capacity (FVC). 75% of the patients developed scoliosis by a median of 13 years, and 40% (n=18) underwent scoliosis surgery. Five children (11%) needed nasogastric feeds and/or gastrostomy.
Schara et al. (65)	SELENON -related muscular dystrophy	Europe/ UK	2008	Clinical findings and course: (n=4) he age of onset of muscle hypotonia, poor head control and delayed motor development was evident during the infantile period . All patients attained ambulation by 13 years of age, 72% developed spinal rigidity, at a mean age of 10 years. All patients had respiratory dysfunction but the age of onset did not correlate with the onset of muscle symptoms and vital capacity ranged from 18% to 65%. 36 of their patients required intermittent nocturnal ventilation at a mean age of 11 years. Muscle biopsies of eight patients showed severe dystrophic changes.
Scoto eta l. (66)	SELENON -related muscular dystrophy	Europe/UK	2011	Clinical findings and course: (N=41) A retrospective cross-sectional study. The mean age at onset in most patients was early childhood (2.7 years). Most were ambulant (95%). Two patients lost ambulation in the late second decade. Outcome: The progressive respiratory dysfunction with nocturnal non-invasive ventilatory support was reported in with late childhood/ adolescent period onset, scoliosis development was 10 years, in most cases

				preceded by the rigidity of the spine. 14 patients (34%) patients had spinal surgery the mean age at surgery was 13.9 years.
Cotta, et al. (79)	LMNA-related muscular dystrophy	South America, Brazil	2014	Clinical findings and course: (N=6) A case series reports, diagnosis made one clinical and genetics, cardiac arrhythmia, muscular weakness, elbow contractures, and intranuclear pseudo inclusions on muscle biopsy
Prigogine et al. (94)	LMNA-related muscular dystrophy	Europe	2010	Clinical findings and course: A case series reports, diagnosis made on clinical and genetics, muscle weakness and wasting affected predominantly axial muscles as well as proximal upper and distal lower extremities. Outcome and clinical course: rapidly developed joint contractures and spine rigidity. creatine kinase was moderately elevated. Muscle biopsy indicated a dystrophic pattern with normal immunochemical findings.
Oliveira, et al (83)	LAMA2 related muscular dystrophy	Europe UK	1993	Clinical descriptive study, the clinical feature, MDC1A is neonatal profound hypotonia, poor spontaneous movements, and respiratory failure, facial muscle weakness, temporomandibular joint contractures, and macroglossia may further impair feeding and can affect speech. In late-onset LAMA2-MD, clinical features vary from early childhood to adulthood. Affected individuals may exhibit muscle hypertrophy and develop a rigid spine syndrome with joint contractures.
Abdel Aleem etal. (81)	LAMA2 related muscular dystrophy	Middle East		Clinical descriptive study: n=17, clinical features, generalized hypotonia, developmental delay, and progressive muscular weakness. Cardiac involvement was rare, clinical course variable
Colombo, etal al. (33)	ACTA1 related myopathy	Europe UK	2014	Retrospective cross-sectional study, n=125 patients, diagnosis made on clinical and genetics, Neonatal/infantile weakness, required respiratory support, and feeding issues Clinical course and outcome: Independent ambulation was achieved in 74.1% of all patients; 62.9% were late walkers. Among ambulant patients, 9% eventually became wheelchair dependent. Scoliosis of variable severity was reported in 40%, with 1/3 of (both ambulant and no ambulant) patients requiring surgery. Bulbar involvement and respiratory insufficiency were present in 46.4%.
Ilkovski et al. (88)	ACTA1 related myopathy	Sydney, Australia.	2005	Clinical descriptive report, mutations in alpha-skeletal actin (ACTA1) underlie several congenital muscle disorders including nemaline myopathy (NM). Nearly all ACTA1-NM patients

				have a healthy cardiac capacity, a reduced foetal movement just before birth.And neonatal weakness, distal muscle involvement.

Table 2. Definitions and clinical descriptions of inclusion groups(11, 19, 20, 33). Differentiating points are in BOLD

Feature	**Collagen VI related dystrophies (46, 47, 49)**	***SELENON*-related CMD** (60, 61, 92, 93)	***LMNA*-related CMD** (71, 94)	***LAMA2* muscular dystrophy** (3, 83)	***ACTA 1*-related myopathy(28, 29)**
Age of onset	Severe early onset (Ullrich) Later onset (Bethlem)	Birth to <2 years	LMNA-CMD <2 Years EDMD2, 2nd–3rd Decade LGMD1B, 3rd–4th Decade.	congenital onset type (MDC1A) to milder late-onset LAMA2-MD	severe neonatal onset to late onset in childhood and adulthood
Weakness distribution	Diffuse (Ullrich) Mild proximal (Bethlem)	Axial weakness, especially neck flexors and proximal regions	LMNA-CMD <2 Years diffuse or dropped head syndrome EDMD2-scapulo/humero/ peroneal. LGMD1B, pelvic/scapular.	Diffuse	Diffuse
Reduced in utero movements	Yes (Ullrich)	Reduced in some	Occasionally in congenital form	Yes- MDCI1A(congenital, severe form)	Yes-severe congenital form
Persistent neck flexor weakness, (Axial involvement	Yes, (Ullrich)	Common and severe	LMNA-CMD <2 Years severe "dropped head" EDMD2, frequent LGMD1B, rare	Yes-severe congenital form	Yes-severe congenital

Hyperlaxity and hypermobility	Yes – especially distal	Moderate distally (less evident than contractures)	Reduced, prominent in the neck	Yes reported	Yes distal
Contractures	Yes – often congenital (Ullrich) progressive, and large joints (Ullrich and Bethlem)	Flexion contractures, at ankles and knees as well upper limbs	LMNA-CMD - Common, distally Then proximally EDMD2- especially contractures LGMD1B rare and late onset	Diffuse and distal contractures	Yes – with time Contractures, or at birth
Ambulation	Rare to walk (Ullrich) Often lost in adulthood (Bethlem)	Most walk independently. Difficulties walking later during life may occur	LMNA-CMD – rarely walk EDMD2- may lose ambulation later life LGMD1B rarely loose ambulation if later life.	Most walk independently. Difficulties walking later during life may occur.	Severe wheelchair-dependent, mild form walk independently
Rigid Spine	Yes – rarely (Ullrich) No (Bethlem)	**Yes – common feature**	LMNA-CMD frequent EDMD2,rare LGMD1B,frequent Maggi etal **(22)**	Yes, reported **(84, 90,94)**	No
Scoliosis	Common in late childhood	Yes. –common complication	LMNA-CMD - frequent EDMD2- frequent LGMD1B rare	Progressive scoliosis starting in childhood	Common in late childhood
Motor deterioration	Severe (Ullrich) Mild (Bethlem	Yes – slowly progressive	Proximal upper limbs and distal lower limbs	Yes – with time, congenital(MDC1A, Axial weakness, difficulties in head control (mainly due to flexor muscles of the neck)	Slow Progressive in congenital, late form- Yes – with time
Respiratory involvement	Early in Ullrich and variable in Bethlem	Restrictive pattern	LMNA-CMD frequent EDMD2- rare LGMD1B - rare	late-onset *LAMA2*-MD, Progressive respiratory insufficiency, Restrictive	Yes, with time, severe in congenital
BIPAP support	Common in 1st or 2nd decade	Yes – in late childhood	LMNA-CMD in a minority EDMD2- rare LGMD1B - rare	Yes-Early in Congenital MDC1A, – late LAMA in late childhood	Yes, with time in late and early in severe congenital
Cardiac involvement	No	Yes – arrhythmias but usually asymptomatic	LMNA-CMD occasionally EDMD2 - DCMO, conduction system defects LGMD1B - DCMO, conduction system defects	Yes- cardiomyopathy with or without cardiac conduction defect can occur in late onset LAMA2 MD	Very rare
Other points	Skin laxity and abnormal scar formation May have torticollis,	Transient feeding difficulties. Mild ptosis, ophthalmoparesis in severe cases.	**Wasting of the cervical axial muscles.** Feeding difficulties. LMNA-CMD– sudden death in 1st decade. No CNS involvement	Facial muscle weakness, and macroglossia, muscle hypertrophy normal intellect,	Prominent facial weakness with or without ptosis, generalised hypotonic ('frog-

	clubfeet. Feeding difficulties. May have skin involvement - extensor surfaces. Normal cognition	**Retained ambulation despite need for BIPAP support can occur.**		**Epilepsy, including of a refractory nature** Structural brain changes.	leg') hyporeflexia, and weakness and dysfunction of the respiratory and bulbar muscles. **The extraocular muscles may be involved**
Serum CK level	Normal – Mildly elevated	Normal - mildly elevated	Normal to moderately raised (<5x normal levels	In both forms markedly raised	Normal to mildly raised
Histology of muscle	Dystrophic changes in congenital onset cases; absence of collagen VI staining in most cases	Myopathic, severe cases dystrophic. Multi minicores/d dystrophic, Mallory-like inclusions	LMNA-CMD, myopathic or dystrophic EDMD2, Nonspecific myopathic. LGMD1B, Nonspecific myopathic	Dystrophic changes, Complete or partial laminin α2 deficiency (	Myopathic feature, Intranuclear rods.
MRI	Concentric pattern of imaging changes. In rectus femoris, central zone of altered signal.	Absence / severe atrophy of semi membranous muscle	Fatty infiltration of medial gastrocnemius and vasti muscles, sparing of rectus femoris	Brain: T2W and FLAIR WM abnormalities	
Nerve involvement	-	-	Non-specific, mainly axonal	Reduced velocities	-
Genetics	COL6A1, COL6A2, COL6A3	SELENON1	LMNA Gene	LAMA2 *gene*	ACTA 1
Inheritance	Ullrich CMD Recessive and de novo dominant mutations Bethlem myopathy-autosomal-dominant	Autosomal-recessive.	Autosomal-dominant, High clinical variability within families	Autosomal recessive pattern	mutations (~90%) are dominant missense changes. 10% Recessive.
Other	May have torticollis, clubfeet. Feeding difficulties. May have skin involvement - extensor surfaces. Normal cognition	Mild ptosis, ophthalmoparesis	LMNA-CMD– sudden death in 1st decade. No CNS involvement	Seizures, structural brain changes, muscle hypertrophy	

CHAPTER 2.0 publication ready report

Delineation of a subgroup of children with connective tissue variant muscular disorders in the Western Cape of South Africa

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ABSTRACT

Background

Muscular dystrophies (MD) and myopathies are a distinct group of clinically and genetically heterogeneous inherited muscle diseases. They cause muscle weakness often with cardiac, pulmonary, and musculoskeletal dysfunction, leading to reduced longevity. MDs and myopathies present across all life stages. Delineation of this condition and specifically the subgroups with additional connective tissue involvement is poorly described in sub-Saharan African populations.

Aim

To delineate the phenotypic, and where possible genotypic expression, of muscular dystrophies and myopathies with connective tissue involvement in an African setting.

Methods

A retrospective cohort study was undertaken of children with muscular dystrophy / myopathy and connective tissue involvement who attend a dedicated neuromuscular service. Patient demographics, diagnosis and clinical profile was collated. Patients were allocated into two groups, congenial /infantile and childhood, based on age of onset. Muscle biopsy characteristics, biochemical findings, and where available, genetic analysis were captured. Based on the combined findings children were categorised into connective tissue variant groups i.e., Collagen 6 related myopathies, Rigid Spine Syndrome (*SELENON* phenotype), *LMNA*-related, *ACTA1* related myopathies, MDC1A, and a subgroup who could not be categorised. Descriptive statistics and categorical variables were compared to evaluate primary study questions. Ethical approval was obtained by the University of Cape Town Human Research Ethics Committee (HREC:549/2019). Families gave informed consent prior to enrolment.

Results

A total of 57 children were reviewed, 50 of whom met the inclusion criteria of connective tissue spectrum in the setting of muscle disease (female to male ratio 1.3:1). There was a predominance in children from African ancestries, followed by those of European descent. 31/50 (62%) presented in the congenital-infantile age period, the remainder presented after 2 years of age. Children with congenital/infantile onset were more likely to lose independent ambulation compared to children with childhood onset (5/8, 62% vs 3/8 38%). Scoliosis complicated the course in 29/50 (58%) children, again affecting congenital/infantile onset children more when compared to the childhood onset group (19/29, 65%, vs 10/29,35%);

(p=0.003), and spinal rigidity was more prevalent in the congenital/infantile onset compared to the childhood group, (8/11 (73%) vs 3/11 (27%)). The childhood group were statistically more at risk of suffering compromising respiratory muscle dysfunction (32/45, 71%, vs 13/45,29%) ;(p=0.04). Genetic diagnosis was available for 9 patients. Based on this and the combined phenotypes n=17 were considered part of the Collagen 6 group, n=7 Rigid Spine Syndrome and n=14 LMNA spectrum, n=3 under the *ACTA1* mutation expression, n=2 LAMA2 and the remaining 7 could not be categorized or did not fall under one of the main groupings.

Conclusion

This study confirmed expression of this subgroup of muscular diseases with connective tissue involvement within the SSA population. The burden of disease from these conditions is across multiple systems and significant, requiring specialized care. Early recognition and referral to neuromuscular centre, would improve the potential outcomes for these children with collaborative multidisciplinary team. Serum creatine kinase (CK) levels and clinical markers such as rigid spine, dropped head, and skin laxity could be used in resource limited settings for probable and possible phenotype.

Key words; Connective tissue spectrum, phenotype, muscular dystrophies, LMNA, Rigid spine Syndrome, LAMA2, ACTA.

Introduction

Muscular dystrophies (MD) are a diverse group of inherited muscle disorders, which are clinically and genetically heterogeneous. They cause muscle weakness often with associated cardiac, pulmonary, musculoskeletal dysfunction leading to reduced longevity. MDs present across all life stages (1). Muscular dystrophies cause progressive muscle wasting (damage) and weakness, especially affecting limb muscles. In some cases, it may affect the muscles of the face and muscles that assist breathing and swallowing (2).
The prevalence of congenital muscular dystrophies (CMD) is incompletely defined. A systematic review in 2016 reported an estimated overall prevalence of 0.99 per 100,000, but data from Africa was lacking(3, 4). Delineation of the genetics of the CMDs are also relatively limited. This is especially the case in Africa, compounded but the lack of expertise and access to high throughput technologies which limits discovery of novel candidate variants which are likely to exist based on the genetic heterogeneity of African populations An additional meta-analysis published the overall pooled prevalence of combined muscular dystrophies was 16.14 (confidence interval [CI],11.21-23.23) per 100,000(5). These estimates are likely to underestimate the true prevalence based on the increasing recognition of the condition and the delineation of an expanded range of clinical phenotypes under the congenital myopathy umbrella(1). The natural history of particular subgroups is reported in only a few cohorts, not all of which were fully genetically delineated (6).

Most MDs are of autosomal recessive inheritance but dominant mutations occur in the setting of de novo mutations e.g. Ullrich CMD (7). MDs are caused by mutations in genes responsible to produce proteins essential for contractile, cytoskeletal, signaling, or enzymatic function within the muscle fibres or extracellular matrix. The explosion in molecular genetics over the past three decades has brought a greater understanding of the biological basis for these disorders and the appreciation that different gene mutations, can cause similar phenotypes (8). Conversely, mutations in one gene can cause very different phenotypes. General themes to MDs are features of muscle fibre degeneration with defective cellular repair, causing to atrophy of these fibres and replacement by fat and connective tissue. This ends in the decline of function and joint contractures in skeletal muscle and in some situations diminished cardiac and visceral (smooth muscle) capacity. In some muscular dystrophies scapular, humeral, and peroneal involvement with early onset of joint contractures and often cardiac involvement (8-10).

The management of muscular dystrophies has largely been symptomatic, with a strong focus on respiratory management, nutritional support, surgical correction of orthopaedic complications, and application of physical and occupational therapy services to maximize motor and respiratory function. For some congenital dystrophies, cardiac management is also necessary (11-13). Several candidate therapies are in preclinical study phases and due to enter the stage of clinical assessment (14). Evaluation of these drugs will hopefully lead to a new era of therapy for a group of previously untreatable and devastating diseases (15-17).

Each MD has distinctive features. Understanding the unique phenotype-genotype correlations, has implications for genetic counselling inclusive of prenatal care, prognostic expectations and aspects of management as well as recruitment for clinical trials (18).

The term “congenital” refers to the pathological process commencing during the fetal period. This may not necessarily correlate with the timing of the clinical manifestation. As such it can be difficult to predict the onset of the clinical symptoms, affected children may develop symptoms at birth, but they may also start to develop weakness, contractures, and delayed motor milestones in the first year of life.(19, 20) In some clinical spectra such as Ullrich CMD and *SEPN1*-related myopathies, the onset can be delayed even beyond the end of the first year (21).

Muscular dystrophies (MD) with connective tissue variant are a group of genetically, clinically, and biochemically distinct entities sharing clinical features characterized by connective tissue involvement. Table 1 and Table 2 summaries the key phenotypic and genetic findings of the more delineated subtypes which include Collagen VI–related muscular dystrophies, *SELENON*-related MD, *LMNA*-related myopathies, LAMA2 mutations and a group with heterogeneous expression associated with ACTA1 mutations.

Collagen VI–related muscular dystrophies(6, 11, 20, 46, 47, 49, 51) constitute a spectrum of severities from the mild Bethlem myopathy, intermediate severity subtype, through to the more severe Ullrich CMD. The disease is identified by a clinical combination of distal laxity, contractures of the proximal joints, skin changes, serum CK which is usually normal or only slightly raised. The most severe form, Ullrich CMD, is characterize by marked weakness concomitant with joint hyperlaxity and contractures at birth. Respiratory insufficiency worsens over time. Muscle biopsy can exhibit variable pathology of myopathic to clearly dystrophic involvement. Diagnosis is usually confirmed by identifying mutations in

one of the three genes encoding one of the alpha chains of collagen type.VI. *COL6A1*, *COL6A2* and *COL6A3*.

The clinical phenotype of ***SELENON*-related CMD.(9, 11, 20, 59-63, 66, 93, 95, 96)** is characterized by weakness and rigidity of the spine with early respiratory insufficiency. Onset of RSS in infancy is caused by mutations in the *SELENON* gene. Motor milestones are delayed but most children with SELENON -related CMD eventually achieve and maintain independent ambulation. Notwithstanding the spine rigidity, scoliosis typically emerges and may need surgical intervention. Restrictive lung disease evolves later in the course usually in the early second decade while still ambulant. Cardiac involvement is not described. The creatine kinase level is typically normal and the muscle biopsy can have mild dystrophic features or have multiminicores, fibre-type disproportion, or myofibrillar changes with Mallory body-like inclusions. Scoliosis and respiratory insufficiency are almost inevitable findings in the second decade, with the possibility of early signs from the first decade.

***Lamin A/C*-associated CMD (*LMNA* gene)**.(11, 20, 71, 74, 78, 82, 83, 94, 97) For the congenital subtype all patients present with weakness and gross motor delays in the first year of life, axial cervical muscle weakness, resulting in a “dropped head” phenotype—a distinct clinical feature of this syndrome. Progression of weakness is variable, but all patients have the selective axial weakness and wasting of the cervicoaxial muscles. A rigid spine syndrome with thoracic lordosis evolves rapidly, followed by development of lower-limb flexion contractures at the knees and ankles. Cardiac dysfunction manifests with cardiac arrhythmias which may progress to cardiac failure. There is rapid progression of respiratory muscle involvement and early ventilatory support needed in the majority of cases. Creatine kinase levels are typically elevated. Muscle biopsy reveals myopathic and/or dystrophic features. Immunohistochemical studies are not helpful as a diagnostic marker. Hence DNA studies are needed for definitive diagnosis by identifying mutations in the *LMNA* gene. *LMNA*-related dystrophy (LMNA-RD) gene is associated with various phenotypes expressing skeletal muscle involvement: limb-girdle muscular dystrophy type 1B (LGMD1B), autosomal dominant Emery-Dreifuss muscular dystrophy (EDMD2); and the congenital muscular dystrophy (LMNA-CMD) described in this section(22). These clinical entities may express with the same *LMNA* mutation and coexist in the same family. The significant clinical overlap supports that these phenotypes should be considered as a continuum in the clinical spectrum of LMNA-RD. The heart is affected in all three entities, with similar features, with age of onset the main differentiating factor. The cardiac

presentation may herald the onset of muscle weakness. Kajino et al described a case with muscle weakness and hypotonia from early childhood and fiber type disproportion (FTD) with no dystrophic changes on muscle biopsy, who was initially diagnosed as having congenital fiber type disproportion (CFTD). Subsequently, he developed cardiac conduction blocks and subsequently a heterozygous mutation in the *LMNA* gene was detected(98).

MDC1A (LAMA) -related muscle disorders(3, 11, 20, 81, 83) are caused by mutations in the *LAMA2* gene, they constitute approximately 50% of total reported CMD cases and are characterized by hypotonia within the first few months of life, with increased levels of serum creatine kinase (CK), multiple joint contractures (23, 24). The *LAMA2* gene spans 65 exons and encodes the laminin-α2 chain, which assembles with laminin-β1 and -γ1 to form laminin-211 in skeletal muscles(25). The mutations within the *LAMA2* gene, lead to complete or partial deficiency of laminin-α2. Patients with a partial deficiency of laminin-α2 exhibit a milder phenotype, while a complete deficiency of the protein always results in severe phenotypes, such as white matter hyperintensities in the brain on T2W MRI (23). MDC1A is predominantly reported in European countries, with a smaller a number of cases described in Asia (26).

With regard to **ACTA1 related myopathies (e.g. Nemaline myopathy, multiminicore and congenital fiber type disproportion (28, 29, 37, 40, 92, 99, 100)** this group illustrates the heterogeneity of expression. Congenital myopathies are clinical and genetic different diseases identified by skeletal muscle weakness varying in severity. Three major forms have been recognised: actin myopathy, intranuclear rod myopathy, and nemaline myopathy. In SA CNM most common form. Internationally central core disease is the most common(27). Mutations in the skeletal muscle actin gene, ACTA1 are responsible for up to 20% of congenital myopathies with a variety of pathologies that includes nemaline myopathy, intranuclear rod myopathy, actin myopathy and congenital fibre type disproportion(37). Nemaline myopathies are a different group of congenital myopathies caused by de novo, dominantly or recessively inherited mutations in at least twelve genes. The genes encoding skeletal α-actin (ACTA1) and nebulin (NEB) are the commonest genetic causes, with in addition alpha- and beta-tropomyosin (*TPM3* and *TPM2*) and troponin T (*TNNT1*)(28) (32). most patients have congenital onset distinguished by muscle weakness and hypotonia, but the spectrum of clinical phenotypes is wide, ranging from severe neonatal presentations to onset of a milder disease in childhood.(28, 29). Among patients with *ACTA1* mutations, the severe form of nemaline myopathy with early onset

muscle weakness, rapid course, and respiratory insufficiency is most frequently reported. Cardiomyopathies have not typically reported in ACTA1 myopathies (35). Sewry et al in their study of patients with nemaline myopathy , noted early onset hypotonia, severe areflexia and proximal muscle weakness, distal contractures, and ligament laxity. Muscle biopsy showed nemaline bodies in a variable proportion of fibres. *ACTA1* was most common gene in their cohort (28). Pou-Serradel et al report a case of nemaline myopathy, severe laxity of the ligaments, and distal laxity. Biopsy showed the presence of large numbers of rods in all muscle fibers stained (99). In a retrospective study of the cases of nemaline congenital myopathy by Botelho et al, the group reported neonatal hypotonia, severe areflexia and proximal muscle weakness, and distal involvement, the rest were diagnosed in teens when they presented with a juvenile form of the disease, with muscle weakness, amyotrophy and scoliosis. Muscle biopsy revealed nemaline bodies in a changeable proportion of fibres. Intranuclear rods were not identified in any case. They concluded that there is great phenotypic and prognostic variety in this disorder(101). Multi-minicore disease is a recessively inherited neuromuscular disorder characterized by multiple cores on muscle biopsy and clinical features of a congenital myopathy. There is marked clinical variability which corresponds to genetic heterogeneity such that the pathological findings are reported with various genes ie not specific to *ACTA1* (36). Ferreiro et al reported a mutation of the SELENON gene, with a classical phenotype and multiminicore disease evident on biopsy. All their patients showed an axial weakness ,scoliosis and respiratory failure; spinal rigidity was evident in some, but not all, patients(92).

Congenital fibre type disproportion (CFTD) is a disorder of skeletal muscle with weakness and hypotonia present at birth or shortly thereafter, with generally slow or absent progression of motor symptoms and frequent skeletal abnormalities, including congenital hip dislocation, joint contractures, foot deformities and kyphoscoliosis(38). Mutations in the α-skeletal actin (*ACTA1*) gene have been identified in severe cases of CFTD, but the molecular mechanisms leading to disproportion in fibre size are unknown. Whether *ACTA1* mutations can also lead to milder cases of CFTD needs to be investigated. (39) Congenital fibre type disproportion (CFTD) is a somewhat nonspecific finding which also occurs with other mutations, is considered a non-progressive or slowly progressive muscle disease. Schessl et al described fiber type disproportion in early collagen VI-related disorders biopsies a non-dystrophic myopathy with predominant fiber atrophy(55).

A positive family history has been reported in about 40% of cases, but the inheritance pattern is not clear. Both autosomal recessive and dominant modes of inheritance have been suggested(40).

Integrin and nesprin related CMD. Nesprin 1, is a component linker of nucleoskeleton and cytoskeleton (LINC) complex and the cytoskeleton. And as such is an integral component of the complex, critical for nuclear positioning and anchorage in skeletal muscle, and is thought to provide an essential link between nuclei and actin. (41, 42) Integrins are transmembrane heterodimers of two different subunits, α and β, associated by non-covalent interactions. The α7 subunit is mainly expressed in skeletal and cardiac muscle, localizing at the sarcolemma, the neuromuscular junctions, and, most prominently, at the myotendinous junctions where it provides an anchorage for laminin α2, conferring mechanical stability and traction resistance to the skeletal muscle fibre(43). Both these subtypes of CMD are rare.

Table 1. Literature review, articles were selected based on those most relevant to study question which provided detailed phenotypic and where possible genotypic descriptions of the typical sub-types

Study/Authors/Title	Study subtype	Country/ Location	Year	Findings of the study
Lampe et al. (46)	Collagen VI-related muscle disorders.	USA	1993	Observational study Clinical findings : Fetal hypokinesia, neonatal hypotonia, weakness, feeding problems and some cases respiratory distress, striking hyperlaxity, especially of the distal joints. Diagnosis depends on typical clinical features and muscle biopsy characteristics. Outcome: The clinical course was usually slowly progression. Outcome: Respiratory involvement ,Ulrich form often results in later loss of ambulation and severe respiratory involvement requiring ventilatory support in the first or second decade of life
Jobsis et al. (47)	Collagen VI-related muscle disorders.	Europe, Netherlands	1999	Clinical findings (n=7): Clinical observational study, early-onset diminished fetal movements, hypotonia, contractures, striking hyperlaxity of distal joints. Diagnosis depends on phenotype and genetics. Outcome: During childhood muscle weakness remained stable but deterioration of walking noted. .
Nadeau et al. (50)	Collagen VI-related muscle disorders.	Europe/ UK	2009	Clinical findings and course (n=13), clinical retrospective observational study, the mean age at onset of symptoms was 12 months. Transient feeding problems might occur in the early neonatal period. (61.5%) achieved independent ambulation at a mean age of 1.7 years. (69.2%) became wheelchair dependent at a mean age of 11.1 years. (70%) nocturnal non-invasive ventilation commenced in the mid adolescent period. Outcome: Further deterioration occurred with development of scoliosis in the first decade of life. All cases were diagnosed based on.
Bonnemann etal. (49)	Collagen VI-related muscle disorders.	USA	2010	Clinical findings and course: Observational study. Early-onset weakness associated with pronounced distal joint hyperlaxity and the early onset or early progression of more proximal contractures. In the most severe cases ambulation is not achieved. In Bethlem myopathy characterized by of early or later onset, but is milder in its manifestations, typically

				allowing for ambulation well into adulthood, whereas typical joint contractures are frequently prominent.
Schessl J etal. (55)	Collagen VI-related muscle disorders.	USA	2008	Muscle biopsies were analyzed from (n= 8) UCMD patients ranging in age from 6 to 30 month. Early histological changes in the muscle of patients with Ullrich collagen muscular dystrophy include dystrophic features (muscle fibres degeneration, regeneration, and inflammation) as the predominant finding.
Moghadaszadeh B etal. (59)	SELENON - related muscular dystrophy	USA	2001	Clinical findings and course: SEPN1-related myopathy (rigid spine syndrome (RSS)) in the majority of cases is associated with generalized muscular weakness, late-onset spinal rigidity, and respiratory insufficiency, most commence non-invasive ventilation in the late childhood period. These patients have normal levels of serum creatine kinase.
Villar-Quile etal. (63)	SELENON - related muscular dystrophy	Europe ,France	2013	large international case series .Clinical findings and course (n=132), severe axial muscle weakness, spinal rigidity, and scoliosis (86.1%, from 8.9 +/- 4 years), with relatively preserved limb strength and ophthalmoparesis in severe cases. All patients developed respiratory failure (from 10.1+/-6 years), 81.7% requiring ventilation while ambulant. Outcome : Reduced the functional capacity, non-invasive nocturnal ventilatory support.
Koul etal. (64)	SELENON - related muscular dystrophy	Oman, Middle East	2015	Clinical findings and course: retrospective observational study (n=12).diagnosis made on clinical and biopsy, Onset of the disease ranged from birth to 18 months of age. , reduced fetal movements, and transient feeding difficulties reported in three patients. Outcome: progression of scoliosis and of contractures reduced the functional capacity in six patients. A restrictive ventilatory defect.
Flanigan KM etal.	SELENON - related muscular dystrophy	USA	2001	Clinical findings and course: (n=4). Case series, all patients had hypotonia and prominent neck weakness in infancy, with the development of spinal rigidity, and early scoliosis in late childhood/adolescence. Muscle biopsy showed minimal, nonspecific myopathic changes. Clinical course: Slowly progressive weakness and early respiratory insufficiency requiring non-invasive ventilation.
Silwal et al.(62)	SELENON -	Europe/ UK	2020	Clinical findings and course: (n=60): Cross-sectional

	related muscular dystrophy			multicentre study, diagnosis made on clinical phenotype and genetics review. The majority of patients (77%) had onset of symptoms in the congenial-infantile range ,early feeding difficulties, hypotonia, poor head/neck control, and developmental delay as common presentations, 60% of the patients were non-ambulant. 83% had progressive respiratory insufficiency, requiring nocturnal non-invasive ventilatory support. Clinical course: A severe reduction in Forced Vital Capacity (FVC). 75% of the patients developed scoliosis by a median of 13 years, and 40% (n=18) underwent scoliosis surgery. Five children (11%) needed nasogastric feeds and/or gastrostomy.
Schara et al. (65)	SELENON - related muscular dystrophy	Europe/ UK	2008	Clinical findings and course: (n=4) he age of onset of muscle hypotonia, poor head control and delayed motor development was evident during the infantile period . All patients attained ambulation by 13 years of age, 72% developed spinal rigidity, at a mean age of 10 years. All patients had respiratory dysfunction but the age of onset did not correlate with the onset of muscle symptoms and vital capacity ranged from 18% to 65%. 36 of their patients required intermittent nocturnal ventilation at a mean age of 11 years. Muscle biopsies of eight patients showed severe dystrophic changes.
Scoto eta l. (66)	SELENON - related muscular dystrophy	Europe/UK	2011	Clinical findings and course: (N=41) A retrospective cross-sectional study. The mean age at onset in most patients was early childhood (2.7 years). Most were ambulant (95%). Two patients lost ambulation in the late second decade. Outcome: The progressive respiratory dysfunction with nocturnal non-invasive ventilatory support was reported in with late childhood/ adolescent period onset, scoliosis development was 10 years, in most cases preceded by the rigidity of the spine. 14 patients (34%) patients had spinal surgery the mean age at surgery was 13.9 years.
Cotta, et al. (79)	LMNA-related muscular dystrophy	South America, Brazil	2014	Clinical findings and course: (N=6) A case series reports, diagnosis made one clinical and genetics, cardiac arrhythmia, muscular weakness, elbow contractures, and intranuclear pseudo inclusions on muscle biopsy
Prigogine et al. (94)	LMNA-related muscular dystrophy	Europe	2010	Clinical findings and course: A case series reports, diagnosis made on clinical and genetics, muscle weakness and wasting affected predominantly axial muscles as well as proximal upper and distal lower

				extremities. Out come and clinical course: rapidly developed joint contractures and spine rigidity. creatine kinase was moderately elevated. Muscle biopsy indicated a dystrophic pattern with normal immunochemical findings.
Oliveira, et al (83)	LAMA2 related muscular dystrophy	Europe UK	1993	Clinical descriptive study, the clinical feature, MDC1A is neonatal profound hypotonia, poor spontaneous movements, and respiratory failure, facial muscle weakness, temporomandibular joint contractures, and macroglossia may further impair feeding and can affect speech. In late-onset LAMA2-MD onset of manifestations range from early childhood to adulthood. Affected individuals may show muscle hypertrophy and develop a rigid spine syndrome with joint contractures.
Abdel Aleem etal. (81)	LAMA2 related muscular dystrophy	Middle East		Clinical descriptive study: n=17, clinical features, generalized hypotonia, developmental delay, and progressive muscular weakness. Cardiac involvement was rare, clinical course variable
Colombo, etal al. (33)	ACTA1 related myopathy	Europe UK	2014	Retrospective cross-sectional study, n=125 patients, diagnosis made on clinical and genetics, Neonatal/infantile weakness, required respiratory support, and feeding issues Clinical course and outcome : Independent ambulation was achieved in 74.1% of all patients; 62.9% were late walkers. Among ambulant patients, 9% eventually became wheelchair dependent. Scoliosis of variable severity was reported in 40%, with 1/3 of (both ambulant and no ambulant) patients requiring surgery. Bulbar involvement and respiratory insuffiency was present in 46.4%.
Ilkovski et al. (88)	ACTA1 related myopathy	Sydney, Australia.	2005	Clinical descriptive report, mutations in alpha-skeletal actin (ACTA1) underlie several congenital muscle disorders including nemaline myopathy (NM). Almost all ACTA1-NM patients have normal cardiac function, a decreased foetal movement just prior to birth. And neonatal weakness, distal muscle involvement.

Table 2. Definitions and clinical descriptions of inclusion groups(11, 19, 20, 33). Differentiating points are in BOLD

Feature	**Collagen VI related dystrophies (46, 47, 49)**	**SELENON-related CMD** (60, 61, 92, 93)	***LMNA*-related CMD** (71, 94)	***LAMA2* muscular dystrophy** (3, 83)	**ACTA 1-related myopathy(28, 29)**
Age of onset	Severe early onset (Ullrich) Later onset (Bethlem)	Birth to <2 years	LMNA-CMD <2 Years EDMD2, 2nd–3rd Decade LGMD1B, 3rd–4th Decade.	congenital onset type (MDC1A) to milder late-onset LAMA2-MD	severe neonatal onset to late onset in childhood and adulthood
Weakness distribution	Diffuse (Ullrich) Mild proximal (Bethlem)	Axial weakness, especially neck flexors and proximal regions	LMNA-CMD <2 Years diffuse or dropped head syndrome EDMD2-scapulo/humero/ peroneal. LGMD1B, pelvic/scapular.	Diffuse	Diffuse
Reduced in utero movements	Yes (Ullrich)	Reduced in some	Occasionally in congenital form	Yes- MDCI1A(congenital, severe form)	Yes-severe congenital form
Persistent neck flexor weakness, (Axial involvement	Yes, (Ullrich)	Common and severe	LMNA-CMD <2 Years severe "dropped head" EDMD2, frequent LGMD1B, rare	Yes-severe congenital form	Yes-severe congenital
Hyperlaxity and hypermobility	Yes – especially distal	Moderate distally (less evident than contractures)	Reduced, prominent in the neck	Yes reported	Yes distal
Contractures	Yes – often congenital (Ullrich) progressive, and large joints (Ullrich and Bethlem)	Flexion contractures, at ankles and knees as well upper limbs	LMNA-CMD - Common, distally Then proximally EDMD2- especially contractures LGMD1B rare and late onset	Diffuse and distal contractures	Yes – with time Contractures, or at birth

Ambulation	Rare to walk (Ullrich) Often lost in adulthood (Bethlem)	Most walk independently. Difficulties walking later during life may occur	LMNA-CMD – rarely walk EDMD2- may lose ambulation later life LGMD1B rarely loose ambulation if later life.	Most walk independently. Difficulties walking later during life may occur.	Severe wheelchair-dependent, mild form walk independently
Rigid Spine	Yes – rarely (Ullrich) No (Bethlem)	**Yes – common feature**	LMNA-CMD frequent EDMD2,rare LGMD1B,frequent Maggi etal (22)	Yes, reported (84, 90,94)	No
Scoliosis	Common in late childhood	Yes. –common complication	LMNA-CMD - frequent EDMD2- frequent LGMD1B rare	Progressive scoliosis starting in childhood	Common in late childhood
Motor deterioration	Severe (Ullrich) Mild (Bethlem	Yes – slowly progressive	Proximal upper limbs and distal lower limbs	Yes – with time, congenital(MDC1A, Axial weakness, difficulties in head control (mainly due to flexor muscles of the neck)	Slow Progressive in congenital, late form- Yes – with time
Respiratory involvement	Early in Ullrich and variable in Bethlem	Restrictive pattern	LMNA-CMD frequent EDMD2- rare LGMD1B - rare	late-onset *LAMA2*-MD, Progressive respiratory insufficiency, Restrictive	Yes, with time, severe in congenital
BIPAP support	Common in 1st or 2nd decade	Yes – in late childhood	LMNA-CMD in a minority EDMD2- rare LGMD1B - rare	Yes-Early in Congenital MDC1A, – late LAMA in late childhood	Yes, with time in late and early in severe congenital
Cardiac involvement	No	Yes – arrhythmias but usually asymptomatic	LMNA-CMD occasionally EDMD2 - DCMO, conduction system defects LGMD1B - DCMO, conduction system defects	Yes- cardiomyopathy with or without cardiac conduction defect can occur in late onset LAMA2 MD	Very rare
Other points	Skin laxity and abnormal scar formation May have torticollis, clubfeet. Feeding difficulties. May have skin involvement - extensor surfaces. Normal cognition	Transient feeding difficulties. Mild ptosis, ophthalmoparesis in severe cases. **Retained ambulation despite need for BIPAP support can occur.**	**Wasting of the cervical axial muscles.** Feeding difficulties. LMNA-CMD– sudden death in 1st decade. No CNS involvement	Facial muscle weakness, and macroglossia, muscle hypertrophy normal intellect, **Epilepsy, including of a refractory nature** Structural brain changes.	Prominent facial weakness with or without ptosis, generalised hypotonic ('frog-leg') hyporeflexia, and weakness and dysfunction of the respiratory and bulbar muscles. **The extraocular muscles may be involved**
Serum CK level	Normal – Mildly elevated	Normal - mildly elevated	Normal to moderately raised (<5x normal levels	In both forms markedly raised	Normal to mildly raised

Histology of muscle	Dystrophic changes in congenital onset cases; absence of collagen VI staining in most cases	Myopathic, severe cases dystrophic. Multi minicores/d dystrophic, Mallory-like inclusions	LMNA-CMD, myopathic or dystrophic EDMD2, Nonspecific myopathic. LGMD1B, Nonspecific myopathic	Dystrophic changes, Complete or partial laminin α2 deficiency (	Myopathic feature, Intranuclear rods.
MRI	Concentric pattern of imaging changes. In rectus femoris, central zone of altered signal.	Absence / severe atrophy of semi membranous muscle	Fatty infiltration of medial gastrocnemius and vasti muscles, sparing of rectus femoris	Brain: T2W and FLAIR WM abnormalities	
Nerve involvement	-	-	Non-specific, mainly axonal	Reduced velocities	-
Genetics	COL6A1, COL6A2, COL6A3	SELENON1	LMNA Gene	LAMA2 *gene*	ACTA 1
Inheritance	Ullrich CMD Recessive and de novo dominant mutations Bethlem myopathy-autosomal-dominant	Autosomal-recessive.	Autosomal-dominant, High clinical variability within families	Autosomal recessive pattern	mutations (~90%) are dominant missense changes. 10% Recessive.
Other	May have torticollis, clubfeet. Feeding difficulties. May have skin involvement - extensor surfaces. Normal cognition	Mild ptosis, ophthalmoparesis	LMNA-CMD– sudden death in 1st decade. No CNS involvement	Seizures, structural brain changes, muscle hypertrophy	

METHODOLOGY

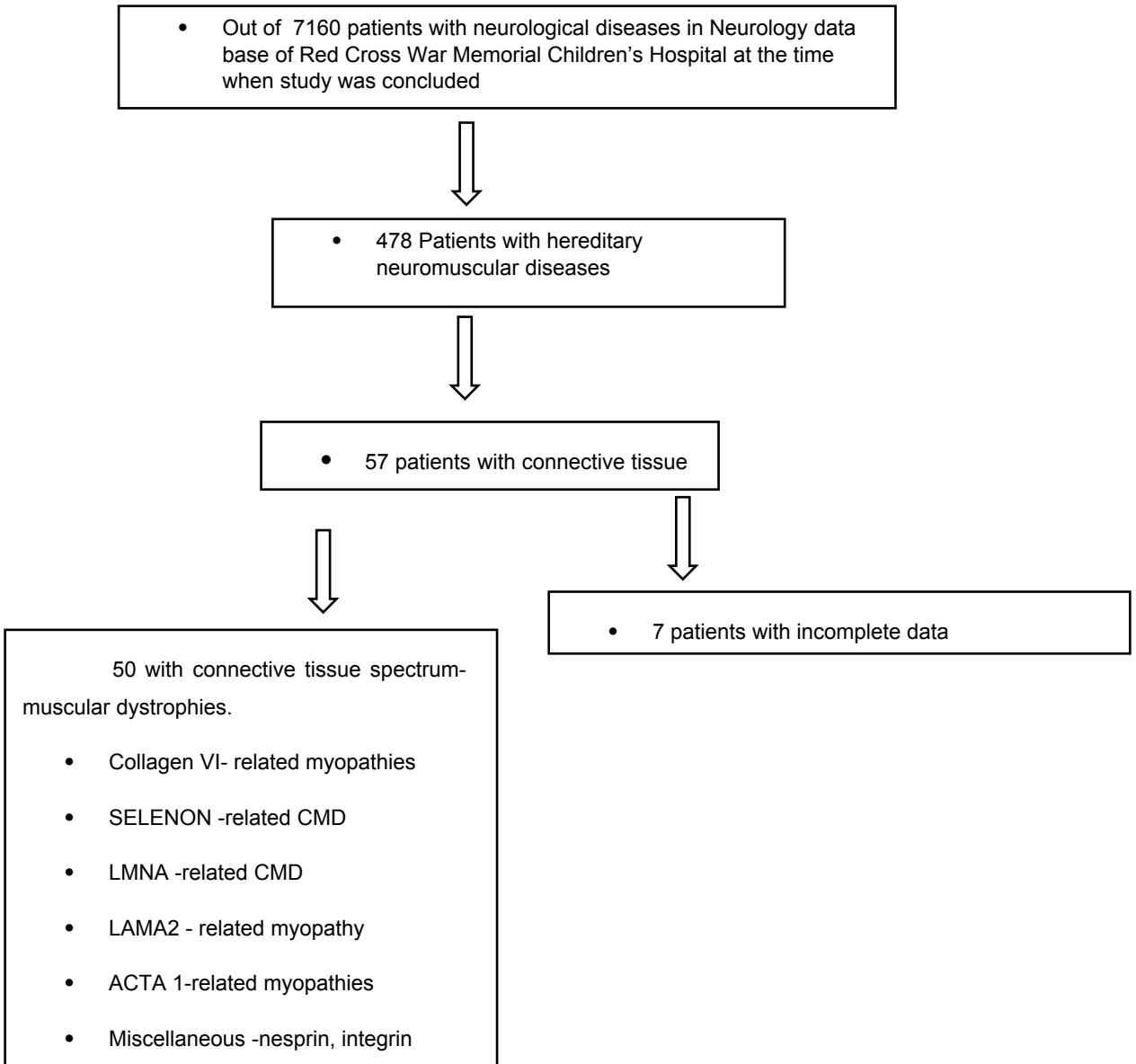
2.1 Study design

A retrospective cohort study was conducted. Infants, children, and adolescents were enrolled from the neurology service with dystrophies / myopathies and connective tissue involvement.

2.2 Settings

The study was undertaken at the Red Cross War Memorial Children's Hospital (RCWMCH), a tertiary public health facility in the Western Cape province of South Africa. The Centre is the largest dedicated children`s hospital in sub-Saharan Africa. Multi-disciplinary interventions are offered from the primary to quaternary level, in-line with international standards of care. The neurology service operates a weekly dedicated neuromuscular clinic with referrals predominantly from the Western Cape and a smaller proportion from further afield. The unit sees some 700 clinic visits annually.

Figure 1. The enrolment of Children with dystrophies with connective tissue variants in the study.



2.3 Study population (Figure 1)

The study population were children and adolescents (0-18 years) residing in the Western Cape province, within the RCWMCH catchment area and outside Cape Town, mostly from rural settings. In addition, a small number were referred from other cities in South Africa where specialist neuromuscular care is not available. Enrolled patients were those diagnosed with dystrophies or myopathies with connective tissue involvement, such that there was either marked joint or ligament laxity and or contracture formation which was out of keeping for the level of mobility. Patients who presented to the neuromuscular service within the neurology department between 2000-2018 were included. The group were identified from the neurology registry (ethics number R013/2013), the neuromuscular clinics, inpatient admissions and referrals. Diagnosis was based on a combination of clinical phenotype, the evolution of disease, creatinine kinases level, muscle biopsy results, cardiac screening, and genetics where available. (19, 20)

2.4 Study participants and sampling methods

2.4.1 Sample size

Out of 478 patients with hereditary neuromuscular diseases who attended the service, 57 had connective tissue spectrum-muscular dystrophies or myopathies. From this group 7 patients were excluded because of incomplete data. A final sample size of 50 children were included in the study analysis.

2.4.2.1 Inclusion criteria

All children aged 0-18 years attending the neuromuscular services at the RXH, with a confirmed diagnosis of muscular dystrophy / myopathy with connective tissue involvement. (19, 20).

2.4.2.2 Exclusion criteria

The study excluded children with inadequate medical records, those with confirmed dystrophinophathies or presumed dystrophy without connective tissue expression as part of their pathology, those with other medical diagnoses such SMA, CMT other forms of hereditary neuropathy or acquired neuromuscular conditions e.g, AIDP, and those whose parents/caregivers who refused to give consent to participate in the study.

2.4.3 Sampling procedures

Parents and/or caregivers were made aware of the research through ongoing verbal explanations via the researcher and the neuromuscular team of clinicians. The researcher was part of the neuromuscular team during his time training as a specialist in paediatric neurology. The researcher identified and approached children who were eligible and their caregivers, explained to them about the research study and sought consent. Those fulfilling the inclusion criteria were interviewed by the researcher in a separate clinic space on the day of their routine clinic attendance whilst awaiting this review i.e., there was no delay in the child's ongoing care. Those who deferred on participation were reassured that this would in no way affect their child's ongoing care. There was 3 stages to the patient assessment, namely the researcher collated the extended data on each child, assessed disease complications, expression and evolution, he also made diagnostic allocations (in line with Table 2 categories), the neuromuscular team met (SR, AN and JW) (blinded to the researcher assessment) and made in independent allocations based on the clinical patient data collected and the medical records, and finally the researcher and supervisor reviewed findings and reached consensus.

2.5 Data collection techniques

Information about the patient's the clinical profile was extracted from the patient's medical records at the RCWMCH as collated by the paediatric neurologist affiliated to the neuromuscular service. For those with missing data, the parent /caregiver was telephonically contacted to provide the information. Further data was collated via the routine clinic visits. Individual data collection was captured using a data extraction tool (data collection tool Appendix 1). Demographics: included child characteristics of age at time of the study, age of onset, gender, ancestry, residence, family characteristics, education level, occupation of primary caregiver, and social welfare support grants. Socioeconomic level were categorized as follows: H0 level (no income/formal employment, H1 level (Household income < R4000 per month/ 300 USD), mid-range H2 (Household income < R8000 per month / 500 USD) level H3 (Household income >R8000 per month/ 500 USD).

Duration of follow-up from time of presentation was assessed to understand the longitudinal capacity of data collected. Based on age at onset of symptoms; patients were stratified into four groups; (0 -1 year), (1 - 2 years), (2-12years) and (12-18 years). As a demonstrator for disease severity and diagnosis, they were further grouped into congenital/ infantile onset and childhood onset groups for clinical descriptions. Muscle weakness distribution and its correlation with the onset of oromotor and respiratory dysfunction, rate of progression, attainment and loss of ambulation, nature of connective tissue involvement was assessed, and family history of confirmed muscle diseases were reviewed. Also, clinical findings including early or subsequent contractures out of keeping with power and mobility, regional muscle atrophy, scapular winging, hypertrophy of calves, rigid spine, scoliosis, and severe axial weakness. Any cardiac involvement was also recorded. Developmental status was evaluated by a developmental paediatrician, all children had access to physiotherapy, occupational therapy, and speech therapy according to their clinical needs.

Investigations

Muscle biopsies were performed in general opportunistically as part of standard care e.g., during gastrostomy insertion or orthopaedic intervention. Muscle biopsy histopathology characteristics included the following parameters: Morphological changes; Variation in myofibre fibres size and shape, atrophic fibres, necrotizing fibres, regenerative fibres, increased endomysial connective tissue and inflammatory infiltrate. Immunohistology included ATPase stains used to stain slow and fast type 1 and type 2 fibres NADH, SDH, COX, modified Gomori to stain ragged fibres, Oil red O for intracytoplasmic lipids. Immunohistochemistry stainable antibody-related technology was used to confirm the presence or absence of muscle proteins, including N dystrophin, C dystrophin, R dystrophin, dystroglycan complex a-dystroglycan, fukutin, laminin, caveolin, Spectrin, Merosin, Dysferlin. Electron microscopy detailed muscle architecture and subcellular components such as mitochondria size and shape, myofibrillar disarray with membrane abnormalities, and subsarcolemmal.

Genomic DNA was typically already extracted from peripheral blood leukocytes or saliva and where possible sent for sequencing. Consent was attained for inclusion of results in the study outcomes. Screening for selected RYR1 mutations was available locally due to the prevalence of its expression in the centronuclear myopathy sub-groups(2).

Based on the collated information of clinical background, genetics and histology the different subtypes of muscular dystrophies were ideally grouped into

(1) Collagen VI-related muscle disorders.

(2) SELENON-related myopathy (rigid spine syndrome (RSS).

(3) LMNA -related myopathy.

(4) LAMA 2 -related muscle disorders.

(5) ACTA1 related myopathies.

(6) Miscellaneous (rare conditions such as integrin and nesprin groups, and the non-specific groups that could not be further categorized**).**

Using the literature review (Table 1) and the collation of key clinical markers (Table 2) diagnostic categorization of the patients was undertaken, by the researcher and independently by the neuromuscular clinicians, following which cases were reviewed for consensus. children were categorised into definite (those with pathogenic genetic confirmation and clinical correlation), probable (based on compatible phenotype some with genetic results which were variants of unknown significance) and possible (based on clinical phenotype alone) diagnoses.

The aim of these results were to understand the landscape of disease expression in this population, compared to other international cohorts, and to establish key clinical markers which could be used as early diagnostic indicators in a resource limited setting.

2.6 Data management and statistical analysis

Exploratory analyses evaluated clinical characteristics of the main outcomes overall and within subgroups, including: The main comparison groups were congenital/infantile-onset vs late childhood onset. Data were collected and entered on a Microsoft Office Excel sheet. Statistical analyses were done in statistical Package for Social Science (SPSS) version 22.0(IBM, United States); all p-values are two-sided, results considered significant when p-values were equal to or less than 0.05. Descriptive statistics were computed describing the sociodemographic characteristics, comorbid states, muscle biopsies characteristics, biochemical characteristics, genetic analysis and diagnosis, age at the onset of symptoms, and age at the time of the study were expressed as mean (standard deviation, SD), were normally distributed (difference median) where skewed (equality of

medians tested with Kruskal-Wallis). Categorical variables were compared with the Chi2 statistic. Summary data was presented in tables and charts. The Mann-Whitney U test used to assess the significance of difference for continuous variables.

2.7 Logistical and ethical considerations

Ethical approval for the study was obtained from the University of Cape Town, Faculty of Health Sciences Research Ethics Committee (HREC REF:549/2019) (Appendix 3), approval was sought from RCWMCH management where the study was conducted (Appendix4). The study complied with the Declaration of Helsinki (2013). Written consent was sought from the parent/legal guardians to participate in the study (Appendix). Where possible verbal assent was also taken from the children, this was mostly children above 8 years of age or according to the child's comprehension of the study. Strict confidentiality and privacy were observed during the interview and data handling. Parents were free to decline participation without any repercussions and assured this would in no way alter their child's care. Data (de-identified) were coded into a secure computer software program.

The study proposal was approved by the RCWMCH Research committee and the University of Cape Town Faculty of Health Science Human Research and Ethics Committee as a sub study of the already ethically approved larger study "Inherited Neuromuscular Disease research: moving towards a definitive molecular diagnosis for each patient Ref Rec R030/2018". The hospital administration also approved the study. Data was stored in a secure setting with access only available to researchers on the study. The patient names were removed from the data collection tool to ensure that confidentiality was maintained, unique numerical identifiers were used instead. The data analysis were combinations of findings and were not in a form to permit individuals to be identified. All parents gave written consent for their children's details to be uploaded into the neurology registry and where possible the children provided assent. Affected children are already regular attendees within the neuromuscular service. The study did not require additional interventions for the children. The study did not result in an additional cost to the hospital or the parents of affected children.

RESULTS

3.1 Introduction

Study findings are addressed under the following 6 subheadings: Sociodemographic, pregnancy/birth, clinical phenotype, biochemical findings, histopathology, and diagnostic categories of the whole group.

50 patients met the study inclusion criteria. (Figure 1)

3. 2 Sociodemographic characteristics

22 (44%) were male and 28(56%) were females (female to male ratio 1.3: 1.0 (p value 0.45). The children were of African ancestry in 34% of cases (n=17), 28% were European ancestry (n=14), 26% were mixed ancestry (n=13) and 12% were Indian (n=6) with no significant difference these categories. Median age of the total group was 11 years of age (range 2 years - 18.5 years).

Most children presented in the congenital-infantile age period (62% (n=31)) compared to 38% (n=19) for the childhood onset period.

65% of the studied group lived in the local metropolis in urban or peri-urban areas. The remaining 35% were from outside Cape Town, mostly from rural settings and a small number from other cities in South Africa where specialist neuromuscular care was not available.

Regarding the maximum level of education of the caregivers, one parent had primary level education, 26(52%) attained secondary school level and 23 (46%) attained tertiary education.

Socioeconomic class distribution among studied group found that majority (59%) were in the most disadvantaged categories, with 38% at H1 level (Household income < 5000 ZAR per month,315 USD, (n=19) and 22% H0 level (no income/formal employment) (n=11). Of the remainder, 36% were categorised as private or H3 (Household income >5000 ZAR per month,315 USD, (n=18). Two patients fell into the mid-range H2 (Household income < 1000ZAR per month,70USD) level (4%).

Forty-nine children (98%) were referred by a paediatrician and one by an orthopaedic specialist.

3.3 Pregnancy and birth history

Eleven children were premature (32-36 weeks n=10; (28-31 weeks,n=1)). All these patients were symptomatic from the birth and categorized into the congenital/infantile onset group, 11/31 (35%).

Polyhydramnios was reported in 12/50 pregnancies (24%) and oligohydramnios in 6/50 pregnancies (12%). All these children were also from the congenital/infantile onset group. In the congenital/infantile onset group, fetal movements in the third trimester of pregnancy were reduced in 21 /31 of the pregnancies (68%). One patient had documented perinatal asphyxia.

3.4. Clinical features of studied group

3.4.1 Motor function

Mild symmetrically reduced facial expression was observed in 11/50 (22%) children most of whom were of congenital-infantile onset. 2/50 (4%) children from the childhood onset had external ophthalmoplegia.

21/50(42%) children had scapular winging, and 7/50 (14%) patients calf pseudohypertrophy, all from the childhood onset group.

22/50(44%) children had mild proximal muscle wasting, and 23/50 (46%) were moderately wasted. Proximal muscle weakness was the main region affected in 36% children 18/50, whilst the remainder were globally weak (n=32) (64%). In 49/50 (98%) of patients the deep tendon reflexes were absent, in one patient the reflexes were normal.

Gower´s manoeuvre of over 20 seconds was observed in 42/50 (84%) children, most of whom were ambulant children from the congenital-infantile group, with static state at the time of testing. “Club feet” or equinus deformities were evident in 14/50 (28%) children, in 3 patients the deformities were noted at birth and in the reminder evolved during the clinical course. 19/50 (38%) children suffered from muscle cramps.

Evolution of the motor dysfunction in the congenital / infantile onset group (n=31), found that 17 patients had a rapidly progressive course, nine patients were initially progressive and then plateaued and five had a static course. In the childhood onset age group (n=19), seven patients had a progressive course, three patients had an initially progressive and subsequently plateaued and 9 patients a static clinical course. A significant difference was

found between the two groups and their clinical progression such that progression was more likely to manifest in the congenital onset group (p value 0.029).

At the time of the study 34/50 (68%) patients had retained independent ambulation, 22/34 (65%) patients were of congenital/infantile onset and 12/34(35%) patients childhood group. 8/50(16%) patients needed assistance to ambulate, three patients (38%) were of congenital /infantile onset, and five patients(62%) childhood onset age. 8/50 (16%) patients lost independent ambulation, 5/8 of whom were congenital onset and 3/8 were childhood onset. Children with congenital/infantile onset were more likely to lose independent ambulation compared to children with childhood onset (5/8, 62% vs 3/8 38%; *p=0.04*). Table 3. The median age at wheelchair use was 4 years of age (± 0.9 SD).

Table 3. Course of disease/Ambulation status of study population

	Independent ambulation	Assisted ambulation	Loss of independent ambulation during clinical course
Congenital/ Infantile onest n=31	22(65%)	3(38%)	5(62%)
Childhood onset n=19	12(35%)	5(62%)	3(38%)
Total (n=50)	34	8	8

Ankle foot orthoses (AFO) were the most common intervention (55%), with the median age of orthotic intervention at 2 years of age ±1.7 SD.

Scoliosis complicated the course in 29/50 (58%) children,19/29 (65%) were of congenital/infantile onset and 10/29 (35%) were childhood onset. As such scoliosis was more prevalent in the congenital/infantile onset compared to the childhood group, (19/29 (65%) vs 10/29 (35%); *p=0.003).* Scoliosis was associated with significant chest wall deformities. Pectus excavatum was noted in 22/29 (76%) children with scoliosis. Scoliosis was rapidly progressive in 18/29(62%), in11/18(61%) were children from the congenital/infantile onset group and 7/18(39%) from the childhood onset group. Spinal rigidity was evident in 11/50 (22%) patients, in 8/11(73%) from the congenital /infantile onset group and 3/11 (27%) were childhood group, as such spinal rigidity was more prevalent in the infantile onset compared to the childhood group, (8/11 (73%) vs 3/11 (27%); *p=0.003).* Most children 22/29 (76%) with scoliosis were managed supportively.

Seven children (24%) required corrective surgery, which occurred without significant post-operative complications in the majority. One child required prolonged intensive care support following complications of malignant hyperthermia including bowel obstruction. Development of scoliosis and non-invasive ventilation intervention with BiPAP, was significantly higher in the late onset childhood group (n=16;100%,P=0.0001).

Arthrogryposis at birth was noted in 6/50 children (12%), all were children of congenital onset. Joint contractures were noted in 43/50 (86%) children during their disease course. The distribution of contractures varied, with involvement of lower extremities hips, knees and ankles predominant in 27 (63%) out of the total 43 patients, in 7(16%) patients distal contracture observed they were congenital -infantile group. In 9/43(21%) had elbow contractures were noted, all from the congenital/infantile group. Achilles tendon contractures occurred 27/50 children (54%). This was severe in 16/27(59%), moderate in nine (33%) and mild in 2/27(7%), predominately from patients in the congenital/infantile group.

Hypermobility of the small joints was significantly higher in the congenital/infantile onset group compared to those of childhood onset (31/50,62% vs 9/50(18%);*P= 0.001*). 36/50(72%) had large joint hypermobility. Hip joint dislocation occurred in 9/50patients (18%), 5 from the congenital onset group and 4 from the childhood onset group. Five (10%) children required transient hip casting and splinting. Pes planus at the time of presentation was noted in 33/50(66%).

3.4.2 Respiratory function:-

Transient respiratory distress was reported at the time of birth in 16/50 (32%) of cases who were subsequently categorised into the congenital onset muscle disease.

28/50(56%) children suffered from recurrent chest infections defined as more than 4 episodes of pneumonia necessitated treatment and admission. 22/28 (78%) of these cases were from the childhood onset group and the remainder from the congenital /infantile group (6/28). As such the childhood group was significantly more affected by recurrent respiratory infections (22/28, 78% vs 6/28, 22%; p=0.03).

90% (45/50) of cases had respiratory muscle weakness, confirmed either clinically by recurrent respiratory infections and / or objectively by respiratory function tests. 32/45 (71%) of cases were in the childhood onset group, and 13/45 (29%) were congenital/infantile

onset group. As such the childhood group were statistically more at risk of suffering compromising respiratory muscle dysfunction 32/45(71%)vs13/45(29%) (p=0.04).

Hypoventilation was evident in 29/45 (64%) of the patients with respiratory muscle weakness as suggested by daytime somnolence and frequent nocturnal arousals (n=4), morning headaches (n=2) and snoring (n=23). FEV1/ FVC ratios had a restrictive pattern in 17/45 (38%), and in two patients an obstructive pattern from children in the adolescent age. This later complication was compatible with complications from repeated chest infections. Abnormal polysomnography was reported in 23/45 (51%) of cases with evidence of hypoventilation. 16/45 patients (36%) with respiratory weakness required assisted non-invasive ventilation via nocturnal Bilevel Positive Airway Pressure (BIPAP) support. Children in the childhood onset group (n=11) were significantly more likely to require BIPAP support, with a smaller proportion from the congenital/infantile group (n=5) (11/16,69%vs5/16,32%; p=0.001). There was significant correlation between use of nocturnal BIPAP support and history of recurrent respiratory infections (R =1, P value 0.02).

3.4.3 Oromotor dysfunction and age of onset

Oromotor dysfunction was found in 41/50 (82%) cases, 32/41 were from the congenital / infantile onset group and in 9/41 from the childhood onset group. As such oromotor dysfunction was significantly higher in the congenital/infantile onset age group, compared to childhood onset (32/41, 78% vs 9/41,22%; *p=0.02*). Seven children required percutaneous gastrostomy (PEG) and Nissen fundoplication, this was predominantly from the congenital onset age group. In the remaining cases, there were transient feeding difficulties managed with nasogastric tube feeding and one with nasojejunal tube support to maintain an adequate nutritional and fluid intake.

In 21/31 (68%) of patients with congenital/infantile onset, oromotor dysfunction was identified when the muscle condition manifested, in the remaining 11/31 (32 %) patients oromotor dysfunction manifested after the recognition of the muscle dysfunction. In 7/9 (78%) of patients in the childhood onset group, oromotor dysfunction was identified when the muscle condition was diagnosed, in the remaining 2/9(22%) patients oromotor dysfunction evolved after the recognition of the muscle dysfunction.

3.4.4 Developmental status among studied group

Developmental assessment was undertaken in all children (n=50) via Griffiths or Molteno assessments and / or based on school placement. In-line with their neuromuscular condition the majority 49/50 (98%) had severe to moderate gross motor delay (n=49) and

one patient had mild gross motor delay. Fine motor delay was evident in 49/50 (98%) of children. Mild to moderate speech delay was reported in 41/50(82%) of the children, with expressive deficit more evident. The remainder had normal speech.

Mild cognitive impairment was found in 26/50 (52%) of children, and the remainder were within normal limits. Hearing was normal in 35/50(70%) children and the remainder 17/50(33%) had mild to moderate hearing impairment. Eleven children had conduction defects, and the six had mild sensorineural hearing impairment, all of whom were of congenital -infantile onset. Two patients (4%) needed hearing aids. Hearing difficulties occurred more frequently in the congenital/infantile onset group, compared to the childhood onset, 12/17(70%) vs 5/17(30%). Developmental delay in fine motor and cognitive domains was statistically more likely to occur in the congenital /infantile onset group (37/51 vs 15/51; *p=0.03).*

3.4.5 The miscellaneous clinical findings

Hyperelastic skin was noted in 43/50 children (86%). 14/50 (28%) children had their course complicated by herniae, 10/14 (71%) were male and four were female. Eight of the male children with inguinal herniae (36%) had undescended testis requiring orchidopexy in two cases, 5 of these children were in the congenital onset group and 3 were from the late onset group. Microcephaly was noted in 8/50 (16%) children and two (4%) had macrocephaly.

Epileptic seizures occurred in 2/50 patients (4%), one of whom had an abnormal electroencephalogram (EEG) and was subsequently diagnosed with a primary seizure disorder, and the other child was diagnosed with recurrent febrile seizures and had a normal EEG. Malignant hyperthermia during anaesthetic procedures occurred in 2/50 (4%) patients, both of whom recovered and were negative for the common *RYR1*variants that are analysed in our setting. These common variants are aligned to children expressing centronuclear myopathy in the local region and as such would not cover the other variants (27). Both affected children had a rigid spine phenotype.

A history of consanguinity was documented in 3/50 (6%) patients, two children were HIV exposed, and history of TB exposure was recorded for 16/50 (32%) children. Family history of premature death of unknown cause was reported in one child. No family history or personal history of episodes of myoglobinuria was documented. Family history of neurological disorders was observed in 4/50 children with developmental delay, speech

delay, hypotonia and undiagnosed spasticity. In one patient there was history of significant joint laxity on the maternal side of the family.

3.5 Biochemical investigations

Serum creatine kinase (CK) levels were normal (26-145 IU) in 42/50 (84%) patients, all of congenital/ infantile onset. In 4/ 50 (8%) children CK levels were 1000 – 2000 IU and in 3/50(6%) children levels were between 2000 - 5000 IU, all were of childhood onset. Two children had levels greater than 5000 IU, also from the childhood onset group.

3.6 Muscle biopsy characteristics

All patients underwent muscle biopsy. Variation in fibre size, shape and atrophy were the commonest findings reported in 26/50 (52%) biopsies. Overtly dystrophic features were evident, including degenerating and regenerating fibres and a prominent build-up of interstitial fibrous tissue in the muscle, necrotising fibres and inflammatory infiltrate were reported in 16/50 biopsies (32%). 12/16 of these dystrophic biopsies were from the congenital/infantile onset group and 4/16 were of childhood onset (12/16, 75% vs 5/16, 25%; p=0.004). As such dystrophic findings were more likely to be seen in the congenital/infantile onset group.

In 9/50(18%) biopsies atrophy of the muscle fibres and fibre type disproportion was noted, 5/9 were from the childhood onset group, and 4/9 were from the congenital/infantile onset group.

Electron microscopic examination showed mitochondrial abnormalities and or prominent mitochondrial size in 26/50 biopsies (52%), myofibrillar disarray with membrane abnormalities were predominant findings in 19/50 (38%) biopsies.

The remaining 5/50(10%) biopsies were reported to be normal.

In most biopsies 45/50(90%) all muscle proteins were detected with standard immunohistochemical stains for dystrophins, sarcoglylcans, beta dystroglycan, emerin, merosin and caveolin found to be present. Of the more challenging stains e.g lamin, calpain, dysferlin these were also present. In 3/50 (6%) biopsies there was abnormal spectrin staining, in 2/51 (4%) biopsies merosin protein was absent (consistent with merosin deficient MD). In one patient with LGMD there was reduced expression of beta sarcoglycan, it was not clear if this was a primary or secondary finding.

3.7 Genetic analysis (Table 4 and 5)

Genetic analysis is not routinely available for our government patients, as such only those with access to private funding or on special motivation, could attain genetic analysis.

Detailed analysis was undertaken in only 12/50 (24%). SELENON gene mutation was undertaken in two patients, with one pathogenic, and other one detected a variant of unknown significance (VUS), both had phenotypes compatible with rigid spine syndrome and biopsies showed mild dystrophic changes with moderately raised CK.

Three children had VUS found in the COL6A region, one in additional had a pathogenic mutation in the RYR1 region, which was more likely to be causative, but the remaining two both with VUS in the COL6A2 region had strong clinical correlation. Without confirmation of pathogenicity via recognition of the variant and or parental studies the mutations remained VUS. Two patients with *LMNA* phenotype, both of childhood onset, had pathogenic variants in *LMNA*.

3/9 (33%) of patients who underwent genetic analysis, carried *ACTA1* gene mutations, two mutations were pathogenic, and one was a VUS, with congenital onset, muscle biopsy detected congenital fibre type disproportion. The phenotype in the children with pathogenic mutations had marked myopathic facies, significant head lag, ligament laxity, early feeding and respiratory challenges. The third child with the VUS in the *ACTA1* region was of congenital /infantile onset, with a phenotype of congenital myopathy, CK was normal and muscle biopsy showed variation in muscle fibres size and mild dystrophic feature.

Table 4. Summary of the patients with genetic confirmation. Two children underwent RYR1 local screens which were negative. Twelve underwent more extensive analysis inclusive of SELENON, COL6A, LMNA, ACTA1 and extended RYR1. Of this group 4 had negative results. This table reports the detailed findings of those with pathogenic results and those with variants of unknown significance (VUS) which could be relevant based on the child's phenotype.

Mutation analysis	**Variant**	**Muscle biopsy findings and immunohistochemistry**	**Phenotypic category**	**Clinical findings**
SELENON n=1 Pathogenic n=1	SELENON gene mutation, c.1315C>T (p.Arg 439) heterozygous pathogenic and c.872G>A (p.Arg291 Gln	Dystrophic, absent selenoprotein staining. Muscle biopsy showed dystrophic changes	Rigid spine spectrum	All early infantile onset, in confirmed case parents were heterozygous carrier for pathogenic variant in SELENON
COL6A n=2 Pathogenic n=0 VUS COL6A2 n= 2	variant C.260C>G p.pro 87Arg, ,(c.2076-2078del (p.Lys692del-homozygous (c.2788C>T (p. Arg930Cys),	Muscle biopsy showed Muscle biopsy showed dystrophic changes, variation in fibre size	Collagen VI related MD	Mild raised CK; supportive muscle biopsy.
LMNA n= 2 Pathogenic n=2 VUS n=1	Pathogenic c.1160T>C (p.Leu387Pro),	Dystrophic feature inflammatory change. Immunohistochemical stains for dystrophins, sarcoglylcans, beta dystroglycans, emerin, merosin and caveolin were present. Electron microscopy found no evidence of central cores or nemaline rods	Rigid spine spectrum-muscular dystrophy/Dropped head	Infantile onset noted to be profoundly hypotonic, early onset respiratory issue and feeding, myopathic . Globally hypotonia with marked head lag. Prominent distal laxity, scoliosis at 2-year, distal laxity , normal CK,
ACTA1 n= 3 Pathogenic n=2 VUS n=1	Pathogenic c.143G>T, p.Gly48Val Pathogenic c.866T>A (p.Ile289Asn VUS c.427T>C (p.Ser143Pro)	Biopsy showed variation in muscle fibre size. CFTD in one	Congenital muscular dystrophy/myopathy Spectrum with ligament laxity.	Congenital/infantile onset, phenotype with a mild congenital muscular dystrophy, mild reduced facial

				expression, the CK level was normal and strong family history of joint laxity.
RYR1 n=1 Pathogenic n=1	c.7784-7791del(p.leu2595Argfs	Muscle biopsy showed variation in muscle fibres size and mild dystrophic feature	Congenital myopathy with ligament laxity	Normal CK, congenital onset with hypotonia and arthrogryposis

Table 5. Summary of the phenotype-genotype correlation. Based on the analysis of the literature review (Table 1) and the key clinical markers (Table 2) the children were categorised into subtypes. These were allocated as definite (where there was pathogenic genetic confirmation), probable (where there was either VUS with strong clinical supporting evidence) or possible (based on clinical phenotype). Where clinical information was not consistent with one of the key groups the child was categorized as unclassified or other.

Diagnostic category	***COL6A N=17 (7 F: 10 M)***	***SELENON N=7 (5 F: 2 M)***	***LMNA N=14 (8 F : 6 M)***	***MDC1A -LAMA N=2 (1 F : 1 M)***	***ACTA1 N=3 (2 F : 1 M)***	**Unclassified / other *N=7***
Definite - pathogenic genetic confirmation	0	1	2	0	2	RYR1 mutation Central core disease n=1
Probable -VUS with strong clinical supporting evidence	2	0	0	0	1	
Possible - phenotype	15	6	12	2	0	Nemaline myopathy n=1 Congenital myopathy n=2 Syndrome undefined n=2 Dystroglycanopathy n=1

Discussion

The aim of this study was to delineate the phenotypic, and where possible genotypic expression, of muscular dystrophies and myopathies, with connective tissue involvement. This is the largest cohort of children with this spectrum of muscle disease to be reported from a sub-Saharan African setting. The analysis confirmed that this range of conditions are expressed in the population across all ancestries. In addition, whilst genetic analysis was lacking for most, it was still possible to clinically phenotype and propose diagnostic categories for most. This had implications for optimimsing clinical care especially of targeted monitoring for respiratory and cardiac compromise. In addition, this study supported preparation for more focused genetic analysis when this becomes available.

Our cohort had a slight but non-significant female predominance (female to male ratio1.3:1), these conclusions differ with others which usually note equal sex ratios (11, 19). A study of patients with rigid spine syndrome by Beggs et al (95), noted a female predominance in their study, similarly this predominance was notable in our study with five out of the seven children with RSS being female.

Children of African ancestry were slightly more predominate in our cohort inclusive of European, Mixed and Indian ancestries, but this lacked significance. The demographics of the region reflect that there are more indigenous African children for the population(109,110). In fact, based on population demographics the proportion of children of European ancestry was greater than expected. This may have reflected reduced disease expression in children of African ancestries or be related to European ancestry children being more socioeconomically advantaged with better pathways to access health care. The neuromuscular service is a specialised resource which supports access for children of high income and low-income families.

65 % of the studied group lived in the local metropolis in urban or peri-urban areas. The remaining 35% were from outside Cape Town, mostly from rural settings and a small number from other cities in South Africa where specialist neuromuscular care was not available, reflecting that a third of the service is supporting patients referred from outside the usual referral locations. This reflects the need for more specialised services.

62% of the children presented in the congenital-infantile age period. These findings are compatible with other studies (46-48, 57, 58). The range in presentation times could reflect delays in referring centres recognising clinical phenotypes.

The high incidence of prematurity, polyhydramnios and oligohydramnios was common amongst the congenital-infantile onset group, and especially reduced fetal movements in the third trimester of pregnancy. These findings are in accordance with other studies (45-47).

Similarly, the transient oromotor dysfunction in the early neonatal period, followed findings in another study (50),

Mild symmetrically reduced facial expression was noted in (22%) of children, all of congenital-infantile onset, this finding compatible with Villar *et al* and other reports, (63, 83, 102-104). Ophthalmoparesis was detected in a few children in our cohort 4%, they were late onset LAMA2 and SELENON this finding similar to other,(63, 83) they reported in few of their patients with severe form.

42% children had scapular winging. 14% of patients had calf pseudohypertrophy, this finding was noted by Oliveira et al, in people late-onset LAMA2-MD (83). But only two children in the group had biopsy supportive of merosin deficiency on immunohistochemical staining.

In line with other reports, 68% of patients had retained independent ambulation (52, 63) . Bonnemann *et al* observed that in the most severe cases of Ullrich CMD ambulation is not achieved, or it may be achieved only for a limited period (49). This finding is in variance with our overall results, 22/34 (65%) of our cohort achieved independent ambulation from the congenital -infantile group, however our report was not restricted to children with Ullrich CMD. Jobsis et al reported that the majority of their patients maintained independent ambulation (47) and Silwal et al noted that 60% of their patients were non-ambulant. These studies illustrated the variance in outcomes according to disease subtypes especially for outcomes of SELENON related muscular dystrophy, versus COL6A and further congenital / early onset forms versus later onset (62). SELENON-related myopathy has been noted to

be more severe and progressive than previously thought, leading to loss of ambulation in 10% of cases,(64) this was similar to our findings.
Scoliosis complicated the course in 58% of children and was statistically more prevalent in the congenital/infantile-onset, the majority were categorised COL6A spectrum and SELENON phenotype. This observation is in conjunction with other studies (62) (92). Scoliosis was correlated with significant chest wall deformities, this finding is similar to other studies (1, 18, 51, 62, 92, 96). Progression of scoliosis was rapidly progressive in 62% children all from the congenital/infantile-onset group, this finding is similar to other cohorts which observed progressive scoliosis(6, 49, 51). In 39% from the childhood-onset group, scoliosis was rapidly progressive, these findings are also consistent other studies, (66, 69, 78, 93, 96), all reported progressive scoliosis during late childhood and established by puberty.

Spinal rigidity was evident in 22% of patients, 73% were from the infantile-onset group, and had SELENON spectrum phenotype. This finding was in concurrence with other studies (62, 66, 105), (71, 94, 106).

Tetreault *et al* noted clinical onset was within the first two years of life with muscle hypotonia, and slow motor function improvement in most. The rigid spine was noted at a mean age of 10 years. All patients developed respiratory impairment. Intermittently nocturnally ventilation is often required for this condition by teenage years. Clinical severity can also be variable with early respiratory failure, as well as lack of ambulation observed. The degree of respiratory involvement is not related to the degree of weakness (54). Scoto *et al* reported that late-onset respiratory insufficiency generally develops by 14 years. Motor abilities remain essentially static over time even in patients with the early presentation this finding similar to our result (66). This was evident in our cohort.

The proportion of children with scoliosis and needing corrective surgery was in line with other reports as well as the ability to tolerate the procedure *(68)*
As noted in children with SELENON-related myopathy, scoliosis development and non-invasive ventilation intervention with BiPAP, was significantly higher in the late-onset childhood group compared to the infantile group (65).

In our cohort hypermobility of the small joints was significantly higher in congenital/infantile-onset group, compared to the childhood-onset. Hyperelastic skin was noted in 86% children.

In line with reports over half the children had recurrent chest infections predominately from the childhood-onset group, related to progressive respiratory muscle weakness (64), (22), (54), (83).

Hypoventilation was evident in 64% of the patients with respiratory muscle weakness. 36% required assisted non-invasive ventilation via nocturnal Bilevel Positive Airway Pressure (BIPAP) support. Children in the childhood onset group were significantly more likely to require BIPAP support, this finding correlates with other cohorts (11, 20, 23, 59, 69, 78, 83).

Oromotor dysfunction in (82%) was predominant amongst children with congenital/infantile, 66% of patients with congenital/infantile-onset. Oromotor dysfunction was identified when the muscle condition manifested for most of the children, and only in the remainder during the subsequent course, this finding is in accord with other studies(46, 50, 78, 83).

As found in our cohort, gross and fine motor delay is recognised as a common complications of this group of patients(11, 20). We found epileptic seizures occurred in a few patients and they were childhood onset, this could have been an independent variable, but seizures are reported in patients with late onset LMNA 2 phenotypes (83).

Malignant hyperthermia during anaesthetic procedures occurred in two patients, both recovered and were negative for the RYR1 common variants that are analysed in our setting. Both affected children had a rigid spine syndrome phenotype whilst rare in this syndrome the complication is reported (68).

Cardiac involvement occurred in a few cases in our study, all of childhood onset, with LMNA phenotype spectrum, and were asymptomatic. Quijano et al noted cardiac arrhythmias in four patients of whom only one of whom was symptomatic (74), similarly Oliveira et al (83).

Serum creatine kinase (CK) levels were normal in the majority of children in our cohort, this aligns with other studies (6, 11, 49, 57). A small number of children with childhood onset/LMNA related myopathy, SELENON and LAMA 2 children, the CK level was moderately elevated as noted by other studies (7, 11, 78, 83).

Variation in fibres size and shape and atrophy were the commonest findings reported in 52% biopsies, this finding is similar to other reports (11, 20). Atrophy of the muscle fibres and fibre type disproportion occurred in the childhood onset group, this finding was comparable to Kajino et al study (98), and in congenital-infantile onset Schessl etal reported predominant fibre atrophy and fibre type disproportion (55). In our cohort we

detected mitochondrial abnormalities and or prominent mitochondrial size as commonest microscopic findings, this nonspecific finding similar to other reports (11, 20).
We observed the challenge of VUS in our setting. The need for parental studies to assist recognition of pathogenesis was also a challenge. It is possible that there are novel variants yet to be identified in our setting and this needs to be considered when using with European ancestry devised panel testing.

We found in a patient with LMNA phenotype, a pathogenic variant in LMNA, c.1160T>C (p.Leu387Pro), this mutation differs from the commonly reported mutation in Late-onset LAMA2-MD variant (c.2461A>C (p.Thr821Pro) (83). Overall pathogenic mutations were identified in five of the study group, with VUS in the larger proportion of those analysed resulting in lack of diagnostic clarity for these patients. For the remainder their categories were based on consistency in other diagnostic markers, as summarized in Table 2, but inevitably without the genetic closure this is potentially flawed as there is overlap in many of the clinical features.

Limitations to the data include that this was a retrospective observational single centre study. However, the neuromuscular service is a regional referral source and as such accepts children from all healthcare sectors, primary to tertiary, government and private. However, children with limited access to healthcare based in remote or very socioeconomically challenged areas may not have been able to attain referrals. Allocating the children into the diagnostic categories was in most cases only possible at a probable or possible level, due to the lack of access to genetic studies. As such these children in most cases can only be hypothesized to comply with the categories based on their clinical phenotypes. The currently available genetic panels are not specific for African populations and may not detect pathogenic variants.

Conclusions

This study confirmed expression of this subgroup of muscular diseases with connective tissue involvement within the SSA population. The burden of disease from these conditions is across multiple systems and significant, requiring specialized care. Early referral to neuromuscular centre, would improve the potential outcomes for these children with collaborative multidisciplinary team.

Only a small percentage of our children had access to genetic testing, this study confirmed challenges to diagnosis reflecting the limited resources even in a tertiary setting. But consistent markers such as CK levels, dropped head, rigid spine, skin laxity and other markers highlighted in Table 2 can be used as clinical indicators to assist categorising probable subtypes. This is important to aid care for those at risk of respiratory and cardiac dysfunction as well as scoliosis and planning for loss of ambulation.

APPENDIX 1

Delineation of the genotype and phenotype of children presenting with myopathies and dystrophies excluding dystrophinophathies in the Western Cape of South Africa.
Master thesis: The subgroup of children with connective tissue variant muscular disorders

Serial /Study number ( )

A .Demographics

I. Sex : Male O Female O
II. Date of birth
III. Date of enrolment in neuromuscular clinic
IV. Age of onset of muscle condition
V. Current age
VI. Current address
VII. Referral source
VIII. Paediatrician O Medical Officer O Nurse O Other specialty, specify O
IX. Rural area O urban O
X. Ancestry:- African O Mixed race /colored O Indian O European O other O
XI. Level of education of care giver :- primary O secondary O university O
XII. Socioeconomic setting level :-H 1 O H2 O H3 O

B. Antenatal history and acute neonatal history

I. Term O preterm O
II. Polyhydramnios Yes O No O
III. Reduced fetal movement Yes O No O , late gestation O
IV. History of perinatal asphyxia Yes O No O

C. Mode of clinical presentation/Symptomatology

- Bulbar muscle / weakness oromotor Yes O NO O Age at onset of symptoms
- Respiratory muscle weakness Yes O No O Age at onset of symptoms
- Proximal muscle weakness Yes O No O Age at onset of symptoms
- Distal muscle weakness Yes O No O Age at onset of symptoms.
- Bulbar, Respiratory, Proximal and Distal involvement Yes O No O
- Rate Degree of progression: Progressive weakness O static weakness
- Respiratory needs:
- Recurrent respiratory infections Yes O No O
- Progressive respiratory deterioration
- Daily time BiPAP Yes O No O if yes, age at commencement
- Nocturnal BiPAP: Yes, O No O if yes, age at commencement
- Intermittent CPAP if yes, age at commencement
- Required invasive assisted ventilation :Yes, O No O age at commencement

- other, specific
- Feeding needs :
- Normal feeding Yes O No O
- History of aspiration Yes O No O
- Required temporary Nasogastric tube feeding : Yes, O No O age at time of intervention
- Required nasojejunal tube placement Yes O No O age at time of intervention
- Required Percutaneous endoscopic gastrostomy only Yes O No O age at time of intervention
- Required Percutaneous endoscopic gastrostomy and Nissen fundoplication Yes O No O age at time of intervention
- Motor function
- Poor head control in infancy Yes O No O
- Delayed motor milestones Yes O NO O specify
- Facial weakness Yes O NO Symmetrical O Asymmetrical
- loss of ambulation Yes O NO O Age at onset of symptoms…….
- Pattern
- Rapidly progressive O slowly progressive O progressive and then static O
- loss of motor skills Yes O NO O Age at onset of symptoms

- Rapidly progressive O Slowly progressive O Progressive and then static
- Central neurology complications
- Seizures Yes O NO O if yes, age at onset and type
- Cognitive impairment Yes O NO O if yes, Mild O moderate O severe O
- Hearing loss/impairment Yes O NO O

D. Past Medical and surgical history

I. History of any surgical intervention Yes O No O
II. Details including type of intervention, age at time of intervention
III. Past history of intensive care admission Yes O No O
IV. Details including reason for admission age at time of admission, number of
V. Admission per year
VI. History of malignant hyperthermia Yes O NO O
VII. History of HIV Yes O NO O
VIII. Past history of tuberculosis Yes O NO O
IX. Other medical health problem .
X. E. Family history of neuromuscular diseases Yes O No O

If yes ,diagnosis and relationship Consanguinity Yes O No O
Premature death in family members Yes O No O
Family history of malignant hyperthermia or any clue Yes O NO O
Episodes of myoglobinuria Yes O NO O

E. Wheelchair Details including type of wheelchair , age at time of intervention.

Orthotic intervention Any orthotic intervention Yes O No O If yes, details, type, age at of

I. Regular Physiotherapy Session Yes O No O
At what started
Where it done
Frequency
Out come in term of improvement of muscular strength
Regular occupational therapy session Yes O No O
At what started
Where it done.
Frequency
Out come in term of activity daily living.
Regular speech and language therapy session Yes O No O
At what started
Where it done.
Frequency
Out come in term of activity daily living

L. Clinical Signs

I. Signs of respiratory distress Yes O NO O
II. Weight –for – age (Z-score) Height -for – age (Z-score)
III. head circumference (Z-score) Weight –for – height (Z-score)

IV. Weight for height moderate wasting O severe wasting O
V. Height for age moderate stunting O severe stunting O
VI. Dysmorphic features Yes O NO O
Detail
VII. Myopathic facies Yes O NO O
VIII. Microcephaly Yes O NO O
IX. Structural eye abnormalities Yes O NO O
X. Specify
XI. Tongue fasciculation Yes O NO O
XII. Sitting Yes O NO O
XIII. Walking Yes O NO O
XIV. Head control Yes O NO O
XV. Deep tendon reflexes present Yes O NO O Grading
XVI. Gower manoeuvre time second
XVII. Distribution of the Weakness and wasting at time of presentation , at what age the weakness start
XVIII. Proximal O Details / muscle group affected
XIX. Distal O Details / muscle group affected
XX. Axial O Axial/proximal O
XXI. Scapular winging Yes O NO O
XXII. North Star Ambulatory Assessment overall score /34
XXIII. 6-minute walk test score
XXIV. Evidence of muscle fibrillation: Yes, O NO O
XXV. Area of tenderness: Yes, O NO O
XXVI. External ophthalmoplegia: (EOM) : Yes, O NO O

M. Evidence of bone and connective tissue components

Hypermobility of large joints Yes O No O
Hypermobility of small joints Yes O NO O
Brighton score
Recurrent joints dislocations Yes O NO O
Flat feet Yes O NO O
club foot Equinus deformities at birth Yes O NO O
Swollen fingers or hands Yes O No O
Joint tenderness Yes O No O
Hyperelastic skin Yes O No O
Hearing impairment Yes O No O
Bluish sclerae Yes O No O myopia Yes O No O
Herniae Yes O No O age at diagnosis ,type
Hip dislocation at birth Yes O NO O
legs that turn outward or appear to differ in length Yes O NO O
limited range of motion Yes O NO O
folds on their legs and buttocks that are uneven when their legs extend Yes O NO O
Delayed gross motor development Yes O NO O
Arthrogryposis at birth Yes O NO O
distribution of joints affected
all limbs O only leg joins O only arms joins O
symmetrical O a symmetrical O
Arthrogryposis multiplex congenita subtype
amyoplasia O, distal arthrogryposis O, and syndromic O
Scoliosis : Yes, O NO O age at diagnosis
Spinal rigidity : Yes, O NO O age at diagnosis
Past history of bone fracture Yes O NO O
Present of acquired joint contracture tight tendon Achilles
Signs of nocturnal hypoventilation
snoring Yes O NO O
Number of pillows
Frequent wake up Yes O NO O Frequency of night arousals
morning headache Yes O NO O
daytime somnolence Yes O NO O

Clinical evidence of cardiomyopathy

Fatigability Yes O NO O

Dyspnea Yes O NO O
Orthopnea Yes O NO O
Lower limb oedema Yes O NO O
frothy sputum Yes O NO O
Epigastric pain Yes O NO O

P. Investigations, at time of presentation

Serum Creatine Kinase Level Other muscle enzymes lactate dehydrogenase, aspartate transaminase
Serum Lactate
Serum potassium
Serum calcium
Serum sodium

Electrocardiograph(ECG) findings

Electromyography (EMG)/NCS findings

Serial echocardiographic findings/features of cardiomyopathy/EF and other parameters

Results of the respiratory function test /or sleep study

Actual FEV1 % FVC FEV1/ FVC .PEFR %.
Histopathologic diagnosis
Availability Yes O No O
Age at muscle biopsy

Histopathologic features

Myopathic O dystrophic O nonspecific myopathic or other O
If myopathic :- specify ,the histopathological subgroup
If dystrophic :- specify ,the histopathological subgroup
Non-specific features
Details of muscle biopsy finding
Immune histochemistry finding
Electron microscopic finding

G-Genetic diagnosis* (Genotype)

Full details of mutated gene found locus and product expression i.e., protein

Phenotype diagnosis* clinical diagnosis

Centro nuclear myopathy O
Muscular dystrophy features O
Myotonic feature O
Myopathy with connective tissue disorder O
Dystrophy with connective tissue disorder O
Nonspecific
Non diagnosed
Subtype
Nemaline congenital myopathy O
Central core myopathy O
Multiminicore myopathy O
Congenital muscular dystrophy O
Congenial Myotonic dystrophy O
Limb girdle muscular dystrophy O Subtype
Facioscapulohumeral muscular dystrophy Subtype O
Emery Dreifuss disease

Quality of Life

Fatigue Yes O No O
Sleep disturbance Yes O No O
Depression Yes O No O
Anxiety Yes O No O
Stigma Yes O No O
Positive affect and wellbeing Yes O No O
Emotional and behavioral Yes O No O
Cognition function intact Yes O No O
Compunction verbal intact Yes O No O
Ability to participate in social role and activities Yes O No O

Services involved in care

Under social worker O psychologist O youth group support O Palliative care plan O Pulmonology team O Orthopaedic team O Genetic counselling O

Activities of Daily Living (ADLs)

Feeding -Total dependent O Totally independent O 50% dependent O
Dressing -Total dependent O Totally independent O 50% dependent O
Bathing-Total dependent O Totally independent O 50% dependent O
Toileting-Total dependent O Totally independent O 50% dependent O
Walking -Total dependent O Totally independent O 50% dependent O
Sleep Pattern
Normal O Abnormal /Discorded breathing O
Number of awakening …… number of pillows……

Schooling

Mainstream school O Special need school O
School Performance Good O average O poor O
Moved school Yes O NO O
Was school supportive? Allowed to reach full potential?
Was a developmental assessment performed? Yes, O NO O

APPENDIX 2

Inherited Neuromuscular database and DNA repository 13 February 2019

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

Title: Inherited Neuromuscular Disease research: moving towards a definitive molecular diagnosis for each patient

INVESTIGATOR: Prof Jo Wilmshurst, Division of Paediatric Neurology, Department of Paediatrics and Child Health, Neuroscience Institute, University of Cape Town.

Co-investigators:

CONTACT NUMBER:

You are being invited to give permission for your child to take part in establishing an inherited Neuromuscular Disease database and DNA repository through a combined project with Groote Schuur Hospital & the University of Cape Town. Your participation is entirely voluntary and you are free to decline to participate. If you say no, this will not affect you or your child negatively in any way whatsoever. The document will inform you about a number of objectives of this research. You may say yes or no to any of them, or to all of them.

What is Neuromuscular Disease (NMD) and why have we asked you for permission for your child to participate?

We use the term 'inherited NMD' to refer to conditions which manifest slowly by affecting the normal functioning of nerves or muscles or the connections between nerves and muscles (nerve-muscle junction). These conditions generally cause very slowly progressive symptoms of weakness in the arms and/or legs over many years. If the condition affects the nerves, then there may be accompanying symptoms of numbness – this is inherited neuropathy. If the condition affects your child muscles, we refer to is as myopathy or muscular dystrophy. There may be members of your family with a similar condition, but your child may be the only person in your family with his or her particular set of symptoms and signs.

The slow onset and progression of NMD suggests that these conditions arise from inherited gene alterations or mutations (see later). Only a few of these inherited conditions can presently be diagnosed and confirmed with a routine genetic test. In Africa and South Africa, we are even more limited than elsewhere in terms of the diagnostic tests which are available. This is because almost all the research presently is being performed in Europe and North America.

Although there are currently very few therapies available for the NMD conditions, most people would agree that having a diagnosis confirmed with a genetic test allows a person to deal with their condition better. With the correct 'label' of a condition a person can read about the specifics of the condition and inform oneself about what to expect in the future. In addition, it may be important for family members who may be at risk and for young adults planning pregnancies, to know the specific gene mutation/variation as tests are available to prevent the possibility of having an affected child, if a parent is at risk. It can therefore be beneficial to have a precise genetic diagnosis. In contrast, persons without a confirmed genetic diagnosis often undergo unnecessary investigations, move from doctor to doctor getting second opinions and spend much time searching for answers.

The aims of this project are to a) collect basic clinical and demographic information about your child's condition and to store that information in a secure database. Your child's name and contact details will be

stored so that we can contact you, but these will be password protected and will only be accessible to prof ANON . We also wish to collect a 10ml blood sample (2 teaspoons of blood) which will be used to extract DNA from your child's and store it in a biobank until we can perform a definitive genetic test. This will be done when we secure the necessary research funding, and you will not be liable for any expense. Once the results become available, which may take months or even years, we will contact you and arrange an appointment to discuss the results.

Genetic testing is expensive. However, we have recently heard that money has been made available by the United Kingdom Medical Research Council (UK-MRC) so that UCT Neurology can work with scientists in London over the next 5 years to comprehensively analyse our patients with NMD. As part of the proposed UK-MRC study we will collect your child's examination data, take your child's blood to extract and store your DNA and proceed with testing the DNA over the next few months. Once your child's genetic test results are available, we will schedule an appointment with yourself and the neurologist team (led by Prof ANON) will explain these results to you. If required, we will schedule an appointment with a genetic counsellor at the Neurogenetics clinics for you, your child, and your family.

Participating in this project will not result in any out-of-pocket expenses to your child's. However, we will not be paying for any transport to the hospital when you give blood or come for the results. We will also request that we can share your child's genetic data in an anonymous way with the UK-MRC as this will help the global community fight NMD and hopefully increase the research effort to find therapies for these diseases. It is important to note that any information shared with other researchers will only be done with a coded sample (so no one will know the identity/name of the person) and after our ethics committee has approved the study.

It is important to note that we will only proceed with genetic tests on your child's DNA once the UCT ethics committee has approved the specific research program.

NMD database

You have been invited to give permission for your child to 'participate' in establishing a database and DNA repository because your child has been diagnosed with either inherited neuropathy or myopathy or muscular dystrophy or anterior horn cell disorder (for example spinal muscular atrophy). These are slowly progressive conditions, which can either present in childhood or adulthood, result in dysfunction of the nerves or muscles due to a faulty protein expression.

While the causes of many NMDs are not known, a great deal of research is being carried out which is discovering previously unknown genetic changes in NMD cases. These genetic changes result in the production of a faulty protein which leads to the development of the nerve or muscle dysfunction. As we uncover genes (and therefore proteins) involved in specific NMD patients it will assist us in the understanding of which proteins are involved in these disease pathways. However, most of this work is presently being performed in European ancestry subjects. Therefore, there is a need for research in African populations who have been neglected in this research area as this will provide a final diagnosis for these patients, but it may also expand our knowledge regarding NMD overall and thereby assist with how we think about developing treatments in the future. There are no specific study related visits, but with this form we ask your permission to keep the details of your child's condition in a database together with your contact details. Your child's name will receive a code and your child's DNA sample will be linked to that code. Because population ancestry is important when we talk about genetic differences, we will ask you to classify yourself and your child according to the SA census racial categories. This may be important because certain conditions may be more frequent in certain populations and therefore, we may need to test for them first in that group. We will contact you when any of your child's results become available which we can discuss at a face-to-face meeting. If you need further genetic counselling after the results have been given, then we will arrange that through the division of human genetics.

What is the NMD database and blood repository all about?

The NMD database refers to storing anonymous data about your child's condition on a computer. Your child's name will be linked to this data and kept in a secure place which will only be available to the principal investigator at UCT/Red Cross War Memorial Children's Hospital. The blood repository refers to taking a blood sample from your child now, giving it a code and storing it in a freezer under that code until we can perform a "gene discovery" research project in the future. We will do our best to store such information and samples in a secure place. Once we can perform studies on your child's blood sample it will only be linked to the code and not your child's name.

What are genes?

Genes are found inside all the cells of the body. They contain coding sequences which can be read by specialized machinery in the cell. This genetic code holds all the instructions necessary for our cells, organs, and systems to function. Once the information from the code is interpreted, this information allows the cell to

make many different proteins. These proteins are the building blocks of the body. If the code has an error or fault (for example the code CAG is altered/mutated to CTG), the cell will not read it correctly and the manufactured protein may be faulty. It is similar to reading a word with a spelling mistake and then it becomes difficult to know what the meaning of the word is.

Most of our genetic code is the same- it holds the basic information that makes us "human". However, the code for some of our genes is unique and gives us the individual characteristics that make us different from one another, such as eye and hair colour. The differences in our genes can also explain why some people are more susceptible to certain diseases than others.

There is new technology available which is able to read the entire code or the "exome", the most important part of a person's entire genetic material. Instructions in the exome tell our cells how to make the right protein components to function properly. Because abnormalities of the information contained in exomes can lead to normal variation (like hair colour) or inherited conditions (like NMD), researchers are using tools such as 'exome sequencing' to search through the genetic code of patients affected by the disease they wish to study and comparing that with exomes from individuals without the disease (controls). By doing this they are able to examine a larger portion of the genetic material than traditional tests and so might discover the cause of NMD in cases where other tests did not. Whole genome sequencing is a newer approach where we look at all the genetic material, not just those pieces coding proteins. This is important as we now know that in between the genes are areas which make 'DNA switches' – these are important for activating certain coding machinery or splicing genes. In other words, the faulty part could be in the switches related to the production of muscle or nerve proteins. In this example not enough proteins are made for the muscle or nerve to remain healthy.

Because the genetic screening of all the genetic material of a person is very expensive and very valuable for other studies elsewhere in the world, researchers are increasingly asking people participating in genetic research whether they would consider allowing their anonymous genetic data to be made available to other researchers. We call this 'data sharing'. This allows us to better understand human variation so that we know what resembles normal variation. Any sharing of data will always be done anonymously (that is coded samples only) and with the approval of our ethics committee. However, you may indicate whether you wish your child's to be part of such a project or not.

If I take part, what will happen?

We will also ask your permission to take a blood sample on your child for later DNA analysis. The blood will be stored at -80°C until we are ready to perform the genetic analysis. This may take a few months to complete. We will ask you to provide consent for storage of the blood and for genetic analysis in the future, pending the approval of the UCT Health Sciences Research Ethics Committee (HREC) of the study. Once we have obtained funding to pursue a DNA gene analysis, we will first need to get ethics approval before the study can proceed. You may give permission for your child to take part in this study or refuse.

May I decide not to take part?

Yes, you may refuse to take part. Your child will remain in the clinic as one of our patients, receive the same care as all the other patients, but we will not collect and store any of your child's data.

Will you benefit from taking part in this research?

The benefit to you is that we aim to provide your child with a specific genetic diagnosis for his or her condition. This information may also be useful for your family members. We will discuss the implications of the diagnosis with you once it is available and will refer you and your child to the genetic counselling service. UCT Neurology will pay for the extraction and storage of the DNA sample and future research funding will pay for the sophisticated gene sequencing analysis.

Who will have access to your medical records or genetic data?

All information collected will be treated as confidential and protected in a room which is locked at all times. If it is used in a publication or thesis, your child's identity will remain anonymous. Only the NMD clinic doctors will have access to information obtained for the purpose of the study. Genetic data will remain coded and the key to the code will remain in the Neurology Division, Red Cross War Memorial Children's Hospital.

Is there anything else that you should know or do?

You can contact the Neurology division, Prof ANON at tel 021-ANON if you have any further queries or encounter any problems.

- You can contact the Health Research Ethics Committee at 021-406 6338 if you have any concerns or complaints that have not been adequately addressed by your study doctor.
- **You will receive a copy of this information and consent form for your own records.**

Declaration by participant
By signing below, I agree for my child …….to take part in a research study entitled
"Neuromuscular Disease Database and DNA repository"
According to the South African national census racial classification system, I would classify my child as
a) black African b) mixed-African ancestry/coloured c) white
d) Indian e) Asian/Chinese f) other_____
I agree for my child to take part in the Neuromuscular Disease Research database/repository

- I have read or had read to me this information and consent form and it has been explained in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is voluntary and I have not been pressurised to take part.

I agree for my child to donate a sample (10mls) of blood which will be stored long-term and may be used for future genetic analysis, if the researchers have obtained approval for that study from the UCT HREC

- I understand that I do not have to give permission for my child to take part in this part of the study

- I agree that the researchers may in the future analyse my child's DNA for possible genes involved in NMD if they have obtained the necessary approval from the UCT HREC

Genomic data sharing:

- I agree that the NMD researchers may share my child's coded or de-identified sample/genetic data with other international researchers as long as the particular study has been approved by the UCT Health Sciences ethics committee (circle)

Yes or No

Signed at (place) …… on (date) …… 20

……	……
Signature of parent of participant	Name of parent of participant
……	……
Signature of NMD clinic doctor	Name of doctor taking consent:

APPENDIX 3

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

FACULTY OF HEALTH SCIENCES
HUMAN RESEARCH ETHICS COMMITTEE
Human Research Ethics Committee

Western Cape Government
Health

DR AN PARBHOO
Manager: Medical Services
Red Cross War Memorial Children's Hospital
Email: Anita.Parbhoo@westerncape.gov.za
Te : +27 21 658 5430 Fax: +27 21 658 5006/5166

12 September 2019

Dr M AM Oshi
Neurology

Dear Dr Oshi,

RESEARCH: RXH: RCC 207

APPENDIX 4

PROJECT TITLE: Delineation of the genotype and phenotype of children presenting with myopathies and dystrophies, excluding dystrophinopathies, in the Western Cape of South Africa.

It is a pleasure to inform you that the hospital Research Review Committee has approved your application to conduct above-mentioned study in the Paediatric Neurology Department at Red Cross War Memorial Children's Hospital.

Yours sincerely,

DR AN PARBHOO
MANAGER: MEDICAL SERVICES

CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

HREC 549/2019

APPENDIX 5- journal author guidelines

NEUROMUSCULAR DISORDERS

Official Journal of the World Muscle Society

DESCRIPTION

This international, multidisciplinary journal includes all features of neuromuscular disorders in childhood and adult life (including muscular dystrophies, spinal muscular atrophies, hereditary neuropathies, congenital myopathies, myasthenias, myotonic syndromes, metabolic myopathies and inflammatory myopathies).

The original articles from all areas of the field:

• Clinical features, such as new clinical articles, case studies of interest, treatment, management, and rehabilitation.

Neuromuscular Disorders is the official journal of the World Muscle Society an international, multidisciplinary, scientific society, dedicated to the advancement and dissemination of information in the field of neuromuscular disorders.

• Studies of animal models relevant to the human diseases.

The journal is aimed at a wide range of clinicians, pathologists, associated paramedical professionals and clinical and basic scientists with an interest in the study of neuromuscular disorders.

The journal is published monthly and aims at rapid publication of high-quality papers of scientific merit as well as general interest to a wide readership. There is also a fast track for rapid publication of new material of outstanding scientific merit and importance.

Neuromuscular Disorders is the official journal of the World Muscle Society an international, multidisciplinary, scientific society, dedicated to the advancement and dissemination of knowledge in the field of neuromuscular disorders.

GUIDE FOR AUTHORS

Types of Paper - Research Articles

There is no limitation on length though most articles are between 2500 and 6000 words long.

These submissions will be managed as a report on a workshop, with the convenor(s) listed as corresponding author(s).

The references should be restricted to those directly relevant to the workshop. Up to three tables, figures or photos may be combined. No abstract is required.

2. A complete list of all PARTICIPANTS will be covered at the end of the report.

3. Full ACKNOWLEDGEMENT.

4. In the source, only the class organizers will be the author(s) of the workshop report.

The list of authors will be covered on the first page of the report, under the title, with a similar format to original papers in the journal.

5. The reports will not be subjected to any peer review.

6. Keywords can be presented for reference.

A brief declaration of interest statement in the title page file or the manuscript file .All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.

A summary declaration of interest statement in the title page file or the manuscript file.

Use of inclusive language

Comprehensive language acknowledges difference, conveys respect to all people, is sensitive to differences, and supports equal possibilities. Content should make no assumptions about the beliefs or commitments of any reader.

Article structure

Papers should be organized in the following format: Abstract (which must consist of a single paragraph only and no sub-headings), Introduction, Materials and Methods, Results and Discussion. Other descriptive headings and sub-headings may be used if appropriate.

Subdivision - numbered sections.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference.

Results

Results should be clear and concise.

Discussion

Explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Similarly, for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

• Title. Concise and informative. Titles are often used in information-retrieval systems.

• Author names and affiliations.

• Corresponding author , correspondence at all stages of refereeing and publication, also post-publication.
Highlights; Highlights are mandatory for this journal as they help increase the discoverability of your article via search engines.
Abstract:
A concise and factual abstract (up to 200 words for full length articles and 150 words for case reports) is required. The abstract should briefly state the purpose of the research, the principal results and major conclusions.
Keywords
Immediately after the abstract, provide a maximum of 6 keywords.
Abbreviations
Define abbreviations that are not standard.
Acknowledgements
Collate acknowledgements in a separate section at the end of the article before the references and do not.
Tables
In text and not as images.
References, Citation in text, references cited in the abstract must be

REFFRENCES

1. Iannaccone ST, Castro D. Congenital muscular dystrophies and congenital myopathies. Continuum (Minneap Minn). 2013;19(6 Muscle Disease):1509-34.
2. Mercuri E, Messina S, Bruno C, Mora M, Pegoraro E, Comi GP, et al. Congenital muscular dystrophies with defective glycosylation of dystroglycan: a population study. Neurology. 2009;72(21):1802-9.
3. Amin M, Bakhit Y, Koko M, Ibrahim MOM, Salih MA, Ibrahim M, et al. Rare variant in LAMA2 gene causing congenital muscular dystrophy in a Sudanese family. A case report. Acta Myol. 2019;38(1):21-4.
4. Campbell MC, Tishkoff SA. African genetic diversity: implications for human demographic history, modern human origins, and complex disease mapping. Annu Rev Genomics Hum Genet. 2008;9:403-33.
5. Mah JK, Korngut L, Fiest KM, Dykeman J, Day LJ, Pringsheim T, et al. A Systematic Review and Meta-analysis on the Epidemiology of the Muscular Dystrophies. Can J Neurol Sci. 2016;43(1):163-77.
6. Bushby KM, Collins J, Hicks D. Collagen type VI myopathies. Adv Exp Med Biol. 2014;802:185-99.
7. Habeeb YK, Al-Bloushi MA, Al-Jumah ES, De Souza TM, Moosa A. Congenital muscular dystrophy in Arab children. J Child Neurol. 2006;21(5):400-5.
8. Carter JC, Sheehan DW, Prochoroff A, Birnkrant DJ. Muscular Dystrophies. Clin Chest Med. 2018;39(2):377-89.
9. Reed UC. Congenital muscular dystrophy. Part I: a review of phenotypical and diagnostic aspects. Arq Neuropsiquiatr. 2009;67(1):144-68.
10. Do TN, Street N, Donnelly J, Adams MM, Cunniff C, Fox DJ, et al. Muscular Dystrophy Surveillance, Tracking, and Research Network pilot: Population-based surveillance of major muscular dystrophies at four U.S. sites, 2007-2011. Birth Defects Res. 2018;110(19):1404-11.
11. Muntoni F, Guicheney P. 85th ENMC International Workshop on Congenital Muscular Dystrophy. 6th International CMD Workshop. 1st Workshop of the Myo-Cluster Project 'GENRE'. 27-28th October 2000, Naarden, The Netherlands. Neuromuscul Disord. 2002;12(1):69-78.
12. Kirschner J. Congenital muscular dystrophies. Handb Clin Neurol. 2013;113:1377-85.
13. Voit T. Congenital muscular dystrophies: 1997 update. Brain Dev. 1998;20(2):65-74.
14. Crudele JM, Chamberlain JS. AAV-based gene therapies for the muscular dystrophies. Hum Mol Genet. 2019;28(R1):R102-R7.
15. Scoto M, Finkel R, Mercuri E, Muntoni F. Genetic therapies for inherited neuromuscular disorders. Lancet Child Adolesc Health. 2018;2(8):600-9.
16. Kemaladewi DU, Bassi PS, Erwood S, Al-Basha D, Gawlik KI, Lindsay K, et al. A mutation-independent approach for muscular dystrophy via upregulation of a modifier gene. Nature. 2019;572(7767):125-30.
17. Rodrigues M, Yokota T. An Overview of Recent Advances and Clinical Applications of Exon Skipping and Splice Modulation for Muscular Dystrophy and Various Genetic Diseases. Methods Mol Biol. 2018;1828:31-55.
18. Hsu YD. Muscular dystrophy: from pathogenesis to strategy. Acta Neurol Taiwan. 2004;13(2):50-8.
19. Muntoni F, Voit T. The congenital muscular dystrophies in 2004: a century of exciting progress. Neuromuscul Disord. 2004;14(10):635-49.
20. Dubowitz V. 22nd ENMC sponsored workshop on congenital muscular dystrophy held in Baarn, The Netherlands, 14-16 May 1993. Neuromuscul Disord. 1994;4(1):75-81.
21. Mercuri E, Muntoni F. The ever-expanding spectrum of congenital muscular dystrophies. Ann Neurol. 2012;72(1):9-17.
22. Maggi L, Carboni N, Bernasconi P. Skeletal Muscle Laminopathies: A Review of Clinical and Molecular Features. Cells. 2016;5(3).
23. Cohn RD, Herrmann R, Sorokin L, Wewer UM, Voit T. Laminin alpha2 chain-deficient congenital muscular dystrophy: variable epitope expression in severe and mild cases. Neurology. 1998;51(1):94-100.

24. Herrmann R, Straub V, Meyer K, Kahn T, Wagner M, Voit T. Congenital muscular dystrophy with laminin alpha 2 chain deficiency: identification of a new intermediate phenotype and correlation of clinical findings to muscle immunohistochemistry. Eur J Pediatr. 1996;155(11):968-76.
25. Colognato H, Yurchenco PD. Form and function: the laminin family of heterotrimers. Dev Dyn. 2000;218(2):213-34.
26. Zhou J, Tan J, Ma D, Zhang J, Cheng J, Luo C, et al. Identification of Two Novel LAMA2 Mutations in a Chinese Patient with Congenital Muscular Dystrophy. Front Genet. 2018;9:43.
27. Wilmshurst JM, Lillis S, Zhou H, Pillay K, Henderson H, Kress W, et al. RYR1 mutations are a common cause of congenital myopathies with central nuclei. Ann Neurol. 2010;68(5):717-26.
28. Sewry CA, Laitila JM, Wallgren-Pettersson C. Nemaline myopathies: a current view. J Muscle Res Cell Motil. 2019;40(2):111-26.
29. North KN, Laing NG, Wallgren-Pettersson C. Nemaline myopathy: current concepts. The ENMC International Consortium and Nemaline Myopathy. J Med Genet. 1997;34(9):705-13.
30. Sanoudou D, Beggs AH. Clinical and genetic heterogeneity in nemaline myopathy--a disease of skeletal muscle thin filaments. Trends Mol Med. 2001;7(8):362-8.
31. Wallgren-Pettersson C, Pelin K, Hilpela P, Donner K, Porfirio B, Graziano C, et al. Clinical and genetic heterogeneity in autosomal recessive nemaline myopathy. Neuromuscul Disord. 1999;9(8):564-72.
32. Clarkson E, Costa CF, Machesky LM. Congenital myopathies: diseases of the actin cytoskeleton. J Pathol. 2004;204(4):407-17.
33. Colombo I, Scoto M, Manzur AY, Robb SA, Maggi L, Gowda V, et al. Congenital myopathies: Natural history of a large pediatric cohort. Neurology. 2015;84(1):28-35.
34. Jungbluth H, Sewry CA, Brown SC, Nowak KJ, Laing NG, Wallgren-Pettersson C, et al. Mild phenotype of nemaline myopathy with sleep hypoventilation due to a mutation in the skeletal muscle alpha-actin (ACTA1) gene. Neuromuscul Disord. 2001;11(1):35-40.
35. Kaindl AM, Ruschendorf F, Krause S, Goebel HH, Koehler K, Becker C, et al. Missense mutations of ACTA1 cause dominant congenital myopathy with cores. J Med Genet. 2004;41(11):842-8.
36. Jungbluth H. Multi-minicore Disease. Orphanet J Rare Dis. 2007;2:31.
37. Feng JJ, Marston S. Genotype-phenotype correlations in ACTA1 mutations that cause congenital myopathies. Neuromuscul Disord. 2009;19(1):6-16.
38. Na SJ, Kim WK, Kim TS, Kang SW, Lee EY, Choi YC. Comparison of clinical characteristics between congenital fiber type disproportion myopathy and congenital myopathy with type 1 fiber predominance. Yonsei Med J. 2006;47(4):513-8.
39. Laing NG, Clarke NF, Dye DE, Liyanage K, Walker KR, Kobayashi Y, et al. Actin mutations are one cause of congenital fibre type disproportion. Ann Neurol. 2004;56(5):689-94.
40. Sobrido MJ, Fernandez JM, Fontoira E, Perez-Sousa C, Cabello A, Castro M, et al. Autosomal dominant congenital fibre type disproportion: a clinicopathological and imaging study of a large family. Brain. 2005;128(Pt 7):1716-27.
41. Rajgor D, Mellad JA, Autore F, Zhang Q, Shanahan CM. Multiple novel nesprin-1 and nesprin-2 variants act as versatile tissue-specific intracellular scaffolds. PLoS One. 2012;7(7):e40098.
42. Rajgor D, Shanahan CM. Nesprins: from the nuclear envelope and beyond. Expert Rev Mol Med. 2013;15:e5.
43. Campbell ID, Humphries MJ. Integrin structure, activation, and interactions. Cold Spring Harb Perspect Biol. 2011;3(3).
44. Norwood FL, Harling C, Chinnery PF, Eagle M, Bushby K, Straub V. Prevalence of genetic muscle disease in Northern England: in-depth analysis of a muscle clinic population. Brain. 2009;132(Pt 11):3175-86.
45. Okada M, Kawahara G, Noguchi S, Sugie K, Murayama K, Nonaka I, et al. Primary collagen VI deficiency is the second most common congenital muscular dystrophy in Japan. Neurology. 2007;69(10):1035-42.

46. Lampe AK, Flanigan KM, Bushby KM, Hicks D. Collagen Type VI-Related Disorders. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, et al., editors. GeneReviews((R)). Seattle (WA)1993.
47. Jobsis GJ, Boers JM, Barth PG, de Visser M. Bethlem myopathy: a slowly progressive congenital muscular dystrophy with contractures. Brain. 1999;122 (Pt 4):649-55.
48. Scacheri PC, Gillanders EM, Subramony SH, Vedanarayanan V, Crowe CA, Thakore N, et al. Novel mutations in collagen VI genes: expansion of the Bethlem myopathy phenotype. Neurology. 2002;58(4):593-602.
49. Bonnemann CG. The collagen VI-related myopathies Ullrich congenital muscular dystrophy and Bethlem myopathy. Handb Clin Neurol. 2011;101:81-96.
50. Nadeau A, Kinali M, Main M, Jimenez-Mallebrera C, Aloysius A, Clement E, et al. Natural history of Ullrich congenital muscular dystrophy. Neurology. 2009;73(1):25-31.
51. Brinas L, Richard P, Quijano-Roy S, Gartioux C, Ledeuil C, Lacene E, et al. Early onset collagen VI myopathies: Genetic and clinical correlations. Ann Neurol. 2010;68(4):511-20.
52. Voermans NC, Bonnemann CG, Huijing PA, Hamel BC, van Kuppevelt TH, de Haan A, et al. Clinical and molecular overlap between myopathies and inherited connective tissue diseases. Neuromuscul Disord. 2008;18(11):843-56.
53. Kirschner J, Hausser I, Zou Y, Schreiber G, Christen HJ, Brown SC, et al. Ullrich congenital muscular dystrophy: connective tissue abnormalities in the skin support overlap with Ehlers-Danlos syndromes. Am J Med Genet A. 2005;132A(3):296-301.
54. Tetreault M, Duquette A, Thiffault I, Bherer C, Jarry J, Loisel L, et al. A new form of congenital muscular dystrophy with joint hyperlaxity maps to 3p23-21. Brain. 2006;129(Pt 8):2077-84.
55. Schessl J, Goemans NM, Magold AI, Zou Y, Hu Y, Kirschner J, et al. Predominant fiber atrophy and fiber type disproportion in early ullrich disease. Muscle Nerve. 2008;38(3):1184-91.
56. Ishikawa H, Sugie K, Murayama K, Ito M, Minami N, Nishino I, et al. Ullrich disease: collagen VI deficiency: EM suggests a new basis for muscular weakness. Neurology. 2002;59(6):920-3.
57. Gara SK, Grumati P, Urciuolo A, Bonaldo P, Kobbe B, Koch M, et al. Three novel collagen VI chains with high homology to the alpha3 chain. J Biol Chem. 2008;283(16):10658-70.
58. Fitzgerald J, Rich C, Zhou FH, Hansen U. Three novel collagen VI chains, alpha4(VI), alpha5(VI), and alpha6(VI). J Biol Chem. 2008;283(29):20170-80.
59. Moghadaszadeh B, Petit N, Jaillard C, Brockington M, Quijano Roy S, Merlini L, et al. Mutations in SEPN1 cause congenital muscular dystrophy with spinal rigidity and restrictive respiratory syndrome. Nat Genet. 2001;29(1):17-8.
60. Merlini L, Granata C, Ballestrazzi A, Marini ML. Rigid spine syndrome and rigid spine sign in myopathies. J Child Neurol. 1989;4(4):274-82.
61. Flanigan KM, Kerr L, Bromberg MB, Leonard C, Tsuruda J, Zhang P, et al. Congenital muscular dystrophy with rigid spine syndrome: a clinical, pathological, radiological, and genetic study. Ann Neurol. 2000;47(2):152-61.
62. Silwal A, Sarkozy A, Scoto M, Ridout D, Schmidt A, Laverty A, et al. Selenoprotein N-related myopathy: a retrospective natural history study to guide clinical trials. Ann Clin Transl Neurol. 2020;7(11):2288-96.
63. Villar-Quiles RN, von der Hagen M, Metay C, Gonzalez V, Donkervoort S, Bertini E, et al. The clinical, histologic, and genotypic spectrum of SEPN1-related myopathy: A case series. Neurology. 2020;95(11):e1512-e27.
64. Koul R, Al-Yarubi S, Al-Kindy H, Al-Futaisi A, Al-Thihli K, Chacko PA, et al. Rigid spinal muscular dystrophy and rigid spine syndrome: report of 7 children. J Child Neurol. 2014;29(11):1436-40.
65. Schara U, Kress W, Bonnemann CG, Breitbach-Faller N, Korenke CG, Schreiber G, et al. The phenotype and long-term follow-up in 11 patients with juvenile selenoprotein N1-related myopathy. Eur J Paediatr Neurol. 2008;12(3):224-30.
66. Scoto M, Cirak S, Mein R, Feng L, Manzur AY, Robb S, et al. SEPN1-related myopathies: clinical course in a large cohort of patients. Neurology. 2011;76(24):2073-8.

67. Viprey M, Trang H, Pomedio M, Bessaci-Kabouya K, Sabouraud P, Cheliout-Heraut F, et al. Early Onset of Sleep-Disordered Breathing in Two Children With SEPN1-Related Myopathies. J Clin Sleep Med. 2017;13(9):1105-8.
68. Jorgensen BG, Laub M, Knudsen RH. Anaesthetic implications of rigid spine syndrome. Paediatr Anaesth. 1999;9(4):352-5.
69. Castets P, Lescure A, Guicheney P, Allamand V. Selenoprotein N in skeletal muscle: from diseases to function. J Mol Med (Berl). 2012;90(10):1095-107.
70. Lescure A, Rederstorff M, Krol A, Guicheney P, Allamand V. Selenoprotein function and muscle disease. Biochim Biophys Acta. 2009;1790(11):1569-74.
71. Bertrand AT, Brull A, Azibani F, Benarroch L, Chikhaoui K, Stewart CL, et al. Lamin A/C Assembly Defects in LMNA-Congenital Muscular Dystrophy Is Responsible for the Increased Severity of the Disease Compared with Emery-Dreifuss Muscular Dystrophy. Cells. 2020;9(4).
72. Mercuri E, Manzur AY, Jungbluth H, Bonne G, Muchir A, Sewry C, et al. Early and severe presentation of autosomal dominant Emery-Dreifuss muscular dystrophy (EMD2). Neurology. 2000;54(8):1704-5.
73. Mercuri E, Poppe M, Quinlivan R, Messina S, Kinali M, Demay L, et al. Extreme variability of phenotype in patients with an identical missense mutation in the lamin A/C gene: from congenital onset with severe phenotype to milder classic Emery-Dreifuss variant. Arch Neurol. 2004;61(5):690-4.
74. Quijano-Roy S, Mbieleu B, Bonnemann CG, Jeannet PY, Colomer J, Clarke NF, et al. De novo LMNA mutations cause a new form of congenital muscular dystrophy. Ann Neurol. 2008;64(2):177-86.
75. Carboni N, Politano L, Floris M, Mateddu A, Solla E, Olla S, et al. Overlapping syndromes in laminopathies: a meta-analysis of the reported literature. Acta Myol. 2013;32(1):7-17.
76. Broers JL, Kuijpers HJ, Ostlund C, Worman HJ, Endert J, Ramaekers FC. Both lamin A and lamin C mutations cause lamina instability as well as loss of internal nuclear lamin organization. Exp Cell Res. 2005;304(2):582-92.
77. Davidson PM, Lammerding J. Broken nuclei--lamins, nuclear mechanics, and disease. Trends Cell Biol. 2014;24(4):247-56.
78. Cotta A, Paim JF, Carvalho E, Valicek J, da Cunha Junior AL, Navarro MM, et al. LMNA-Related Muscular Dystrophy with Clinical Intrafamilial Variability. J Mol Neurosci. 2019;69(4):623-7.
79. Philpot J, Sewry C, Pennock J, Dubowitz V. Clinical phenotype in congenital muscular dystrophy: correlation with expression of merosin in skeletal muscle. Neuromuscul Disord. 1995;5(4):301-5.
80. Geranmayeh F, Clement E, Feng LH, Sewry C, Pagan J, Mein R, et al. Genotype-phenotype correlation in a large population of muscular dystrophy patients with LAMA2 mutations. Neuromuscul Disord. 2010;20(4):241-50.
81. Abdel Aleem A, Elsaid MF, Chalhoub N, Chakroun A, Mohamed KAS, AlShami R, et al. Clinical and genomic characteristics of LAMA2 related congenital muscular dystrophy in a patients' cohort from Qatar. A population specific founder variant. Neuromuscul Disord. 2020;30(6):457-71.
82. Nelson I, Stojkovic T, Allamand V, Leturcq F, Becane HM, Babuty D, et al. Laminin alpha2 Deficiency-Related Muscular Dystrophy Mimicking Emery-Dreifuss and Collagen VI related Diseases. J Neuromuscul Dis. 2015;2(3):229-40.
83. Oliveira J, Parente Freixo J, Santos M, Coelho T. LAMA2 Muscular Dystrophy. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Mirzaa G, et al., editors. GeneReviews((R)). Seattle (WA)1993.
84. Wallgren-Pettersson C, Bushby K, Mellies U, Simonds A, Enmc. 117th ENMC workshop: ventilatory support in congenital neuromuscular disorders -- congenital myopathies, congenital muscular dystrophies, congenital myotonic dystrophy and SMA (II) 4-6 April 2003, Naarden, The Netherlands. Neuromuscul Disord. 2004;14(1):56-69.

85. Bentley G, Haddad F, Bull TM, Seingry D. The treatment of scoliosis in muscular dystrophy using modified Luque and Harrington-Luque instrumentation. J Bone Joint Surg Br. 2001;83(1):22-8.
86. Sewry CA, D'Alessandro M, Wilson LA, Sorokin LM, Naom I, Bruno S, et al. Expression of laminin chains in skin in merosin-deficient congenital muscular dystrophy. Neuropediatrics. 1997;28(4):217-22.
87. Hayashi YK, Tezak Z, Momoi T, Nonaka I, Garcia CA, Hoffman EP, et al. Massive muscle cell degeneration in the early stage of merosin-deficient congenital muscular dystrophy. Neuromuscul Disord. 2001;11(4):350-9.
88. Ilkovski B, Clement S, Sewry C, North KN, Cooper ST. Defining alpha-skeletal and alpha-cardiac actin expression in human heart and skeletal muscle explains the absence of cardiac involvement in ACTA1 nemaline myopathy. Neuromuscul Disord. 2005;15(12):829-35.
89. Laing NG, Dye DE, Wallgren-Pettersson C, Richard G, Monnier N, Lillis S, et al. Mutations and polymorphisms of the skeletal muscle alpha-actin gene (ACTA1). Hum Mutat. 2009;30(9):1267-77.
90. Schroder JM, Durling H, Laing N. Actin myopathy with nemaline bodies, intranuclear rods, and a heterozygous mutation in ACTA1 (Asp154Asn). Acta Neuropathol. 2004;108(3):250-6.
91. Moreno CAM, Abath Neto O, Donkervoort S, Hu Y, Reed UC, Oliveira ASB, et al. Clinical and Histologic Findings in ACTA1-Related Nemaline Myopathy: Case Series and Review of the Literature. Pediatr Neurol. 2017;75:11-6.
92. Ferreiro A, Quijano-Roy S, Pichereau C, Moghadaszadeh B, Goemans N, Bonnemann C, et al. Mutations of the selenoprotein N gene, which is implicated in rigid spine muscular dystrophy, cause the classical phenotype of multiminicore disease: reassessing the nosology of early-onset myopathies. Am J Hum Genet. 2002;71(4):739-49.
93. Goto I, Nagasaka S, Nagara H, Kuroiwa Y. Rigid spine syndrome. J Neurol Neurosurg Psychiatry. 1979;42(3):276-9.
94. Prigogine C, Richard P, Van den Bergh P, Groswasser J, Deconinck N. Novel LMNA mutation presenting as severe congenital muscular dystrophy. Pediatr Neurol. 2010;43(4):283-6.
95. Koul R, Sankhla D, Al-Jahdhami S, Mani R, Rahim RA, Al-Yaarubi S, et al. Rigid Spine Syndrome among Children in Oman. Sultan Qaboos Univ Med J. 2015;15(3):e364-9.
96. Fishchenko V, Tarabanova LV. [Rigid spine syndrome in children and adolescents]. Zh Nevropatol Psikhiatr Im S S Korsakova. 1986;86(3):355-7.
97. Durbeej M. Laminin-alpha2 Chain-Deficient Congenital Muscular Dystrophy: Pathophysiology and Development of Treatment. Curr Top Membr. 2015;76:31-60.
98. Kajino S, Ishihara K, Goto K, Ishigaki K, Noguchi S, Nonaka I, et al. Congenital fiber type disproportion myopathy caused by LMNA mutations. J Neurol Sci. 2014;340(1-2):94-8.
99. Pou-Serradell A, Navarro C. [A case of nemaline myopathy studied over a period of nine years (author's transl)]. Rev Neurol (Paris). 1978;134(4):295-302.
100. Agrawal PB, Strickland CD, Midgett C, Morales A, Newburger DE, Poulos MA, et al. Heterogeneity of nemaline myopathy cases with skeletal muscle alpha-actin gene mutations. Ann Neurol. 2004;56(1):86-96.
101. Botelho CH, Carod-Artal FJ, Kalil RK. [Nemaline congenital myopathy:clinical features and histopathological findings in nine patients]. Rev Neurol. 2001;32(4):309-14.
102. Jones KJ, North KN. External ophthalmoplegia in neuromuscular disorders: case report and review of the literature. Neuromuscul Disord. 1997;7(3):143-51.
103. Salih MA, Al Rayess M, Cutshall S, Urtizberea JA, Al-Turaiki MH, Ozo CO, et al. A novel form of familial congenital muscular dystrophy in two adolescents. Neuropediatrics. 1998;29(6):289-93.
104. Voit T, Parano E, Straub V, Schroder JM, Schaper J, Pavone P, et al. Congenital muscular dystrophy with adducted thumbs, ptosis, external ophthalmoplegia, mental retardation and cerebellar hypoplasia: a novel form of CMD. Neuromuscul Disord. 2002;12(7-8):623-30.
105. Sponholz S, von der Hagen M, Hahn G, Seifert J, Richard P, Stoltenburg-Didinger G, et al. Selenoprotein N muscular dystrophy: differential diagnosis for early-onset limited mobility of the spine. J Child Neurol. 2006;21(4):316-20.

106. Maraldi NM, Capanni C, Cenni V, Fini M, Lattanzi G. Laminopathies and lamin-associated signaling pathways. J Cell Biochem. 2011;112(4):979-92.
107. Ben Hamida M, Hentati F, Ben Hamida C. [Multiminicore disease in a rigid spine syndrome]. Rev Neurol (Paris). 1987;143(4):284-9.
108. Beggs AH, Agrawal PB. Multiminicore Disease - RETIRED CHAPTER, FOR HISTORICAL REFERENCE ONLY. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Mirzaa G, et al., editors. GeneReviews((R)). Seattle (WA)1993.
109. Statistics South Africa's. Mid-year population estimates, 2020. Report No.: P0302.
110. Elsenburg. A profile of the western cape province: Demographics,poverty, income, inequality and unemployment from 2000 till 2007. February 2009.