Muscle diseases with damaged sarcomeres - causes and consequences

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ABSTRACT

Muscle diseases, also called myopathies, are usually defined as diseases where the pathology is confined to the muscle itself. This excludes diseases caused by structural abnormalities in the peripheral nerve, from the anterior horn cell to the neuromuscular junction. Much effort has been made to elucidate the pathogenesis of skeletal muscle diseases that result from mutations in sarcomeric and associated proteins, highlighting their importance in normal muscle structure and function. The short-term goals in this field are to determine the remaining causative genes behind the skeletal muscle diseases and to learn more about the pathogenesis behind these diseases. The long-term goals are to develop more specific therapy in the future.

In paper I we investigated two children with nemaline myopathy and identified two *de novo* heterozygous mutations not previously described in the skeletal α-actin gene (*ACTA1*). The marked variability in clinical features in spite of similar muscle pathology in early childhood was demonstrated. The severe muscle atrophy with replacement of fat and connective tissue found in one of the patients demonstrated that nemaline myopathy might be progressive in some cases.

In paper II we investigated a mother and daughter with similar clinical but different morphological features, nemaline myopathy and cap disease. We identified a heterozygous missense mutation in the β-tropomyosin gene, *TPM2,* the first mutation to be found in cap disease. We concluded that candidate genes in cap disease ought to be found within the genes encoding for sarcomeric proteins, especially those previously associated with nemaline myopathy and that mutations in *TPM2* might be a common cause of cap disease.

In paper III we investigated three unrelated patients and identified three *de novo* heterozygous mutations in *TPM2*: a three–base pair deletion in-frame, a three-base pair duplication in-frame, and a missense mutation. The hypothesis that mutations in *TPM2* are a common cause of cap disease was confirmed. In muscle biopsy specimens, a coarse-meshed and irregular intermyofibrillar network was found. These specific pathological findings may be clues towards a correct diagnosis and indicate that the pathogenesis involves defective assembly of myofilaments.

In paper IV we had the opportunity to investigate one of the original cases of cap disease. In this patient we found a *de novo* heterozygous missense mutation in *TPM3*. The observation that cap disease, like nemaline myopathy, is associated with mutations in *TPM2* as well as in *TPM3* and shows similar clinical presentation supports our concept that cap disease is related to nemaline myopathy and all genes encoding components of the sarcomeric thin filament should be considered as candidate genes in patients with cap disease.

In paper V we investigated seven individuals from two apparently unrelated families with a dominantly inherited adult-onset myopathy with early respiratory failure. All patients had muscle weakness in the pelvic girdle, neck flexors and trunk muscles together with prominent calf hypertrophy. Muscle histopathological features included eosinophilic deposits and extensive myofibrillar lesions with marked Z-disk alterations. Genetic analysis with array data using SNP markers demonstrated that the affected individuals shared a large haplotype on chromosome 2q31, including the giant titin gene (*TTN)*. Further studies include the investigation of the *TTN* gene and other genes of interest in this region.

This study has deepened the understanding of inherited myopathies associated with damaged sarcomeres by describing new mutations in causative genes, which in the end could lead to new therapy strategies.

Key words: Congenital myopathies, nemaline myopathy, cap disease, hereditary myopathy with early respiratory failure, myofibrillar myopathy, *ACTA1, TPM2, TPM3, TTN.*

LIST OF PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I **Ohlsson M**, Tajsharghi H, Darin N, Kyllerman M, Oldfors A. Follow-up of nemaline myopathy in two patients with novel mutations in the skeletal muscle alpha-actin gene (*ACTA1*). Neuromuscul Disorder 2004;14:471-475.

II Tajsharghi H, **Ohlsson M**, Lindberg C, Oldfors A. Congenital myopathy with nemaline rods and cap structures caused by a mutation in the beta-tropomyosin gene (*TPM2*). Arch Neurol 2007;64(9):1334-1338.

III **Ohlsson M**, Quijano-Roy S, Darin N, Brochier G, Lacène E, Avila-Smirnow D, Fardeau M, Oldfors A, Tajsharghi H. New morphologic and genetic findings in cap disease associated with β-tropomyosin (*TPM2*) mutations. Neurology 2008;71:1896-1901.

IV **Ohlsson M**, Fidzianska A, Tajsharghi H, Oldfors A. *TPM3* mutation in one of the original cases of cap disease. Neurology 2009;72:1961-1963.

V **Ohlsson M**, Brådvik B, Lindberg C, Tajsharghi H, Martinsson M, Oldfors A. Familial myopathy with early respiratory failure and sharing of a large haplotype at chromosome 2q31. Manuscript.

Amor víncit omnia

Gränslös kärlek är ett vapen med makalös kraft. Den är "summum bonum" i livet. Den finns som ett viktigt kännetecken hos den som är modig. Egentligen är det bara kärleken som betyder något. Den kommer inte inom räckhåll för den som är feg. Den är ingen livlös teori utan en levande och livgivande kraft. Kärleken är hjärtats värdefullaste skatt.

Mahatma Gandhi (1869-1948)

To Jonas and Niklas

CONTENTS

ABBREVIATIONS

AMP	Adenosine monophosphate
ATP	Adenosine 5´-triphosphate
cDNA	Complementary DNA
CFTD	Congenital fiber type disproportion
CK	Creatine kinase
CM	Congenital myopathy
CTG	Cytosine-thymidine-guanine
DA	Distal arthrogryposis
DM1	Myotonic dystrophy type 1
DM	Dermatomyositis
DMPK	Dystrophia myotonica protein kinase
DNA	Deoxyribonucleic acid
EM	Electron microscopy
EMG	Electromyography
F-actin	Filamentous actin
FSH	Facioscapulohumeral muscular dystrophy
G-actin	Globular actin
IBM	Inclusion body myositis
IIM	Idiopathic inflammatory myopathy
IM	Inflammatory myopathies
LGMD	Limb-girdle muscular dystrophy
MD	Muscular dystrophy
MDa	Mega dalton
mtDNA	Mitochondrial DNA
MFM	Myofibrillar myopathy
MRC	Medical Research Council
MRI	Magnetic resonance imaging
MyHC	Myosin heavy chain
MyLC	Myosin light chain
MyBP	Myosin binding protein
NADH	Nicotineamid adenine dinucleotide hydrogenase
NCAM	Neural cell adhesion molecule
nDNA	Nuclear DNA
NM	Nemaline myopathy
PAS	Periodic acid and Schiff’s reagent
PCR	Polymerase chain reaction
PM	Polymyositis
RFLP	Restriction fragment length polymorphism
SNP	Single nucleotide polymorphism
Tm	Tropomyosin
TnC	Troponin C
TnI	Troponin I
TnT	Troponin T
ZASP	Z-band alternatively spliced PDZ motif protein

INTRODUCTION

Muscle diseases

The term "Neuromuscular disorders" is a comprehensive description of disturbances in the motor unit, which consists of the anterior horn cell of the spinal cord, the peripheral nerve, the neuromuscular junction and the muscle fiber. Often the different neuromuscular disorders produce the same clinical features with muscle weakness and hypotonia regardless of which part is primarily affected. The diagnosis is therefore mainly based upon the clinical features together with histopathological findings and electromyographic (EMG) studies of the muscle. The latter can distinguish between neurogenic and muscle diseases. Muscle diseases, also called myopathies, are usually defined as diseases where the pathology is confined to the muscle itself. This excludes diseases associated with structural abnormalities in the peripheral nerve, from the anterior horn cell to the neuromuscular junction. Myopathies can moreover be divided into inherited or acquired myopathies. There are different classifications of inherited myopathies and the classification used here is based on a gene table from Neuromuscular Disorders [1].

Inherited myopathies

1. Muscular dystrophies
2. Congenital muscular dystrophies
3. Myotonic dystrophies
4. Congenital myopathies
5. Distal myopathies
6. Channelopathies
7. Metabolic myopathies
8. Other myopathies

Acquired myopathies

1. Inflammatory myopathies
2. Endocrine myopathies
3. Toxic and drug-induced myopathies
4. Myopathies associated with systemic illness

Inherited myopathies

The muscular dystrophies (MD) are a clinically and genetically heterogeneous group of muscle disorders that may present in early infancy, childhood or adulthood with muscle weakness and muscle wasting in common [2]. They are typically progressive. Several MD are caused by defects in genes encoding sarcolemmal proteins, others are caused by defects in nuclear membrane proteins or enzymes. Morphologically, these disorders are characterized by diffuse variation in the size of muscle fibers, necrosis, fiber regeneration and fibrosis. The most common MD is Duchenne muscular dystrophy, an X-linked recessive disease caused by lack of dystrophin, a protein associated with the plasma membrane [3, 4]. Facioscapulohumeral muscular dystrophy (FSH) is another common MD [5]. It has been mapped to a sub-telomeric region of chromosome 4q35 and the causal genetic defect was identified as a deletion of an integral number of 3.3-kb polymorphic repeats, D4Z4 [6, 7]. In limb-girdle muscular dystrophies (LGMD) the defective proteins are localized to various compartments in the muscle fiber, including the sarcolemma, nuclear envelope, sarcoplasm and components of the sarcomere [2].

The congenital muscular dystrophies (CMD) encompass a heterogeneous group of genetically, clinically and biochemically distinct entities, which applies to infants presenting with muscle weakness at birth or the first few months of life and where muscle biopsy shows dystrophic myopathy [8]. The CMD may also be associated with contractures or hypermobility of various joints. In some of the variants, significant central nervous system and ocular involvement are present. CMD

biochemical types include various abnormalities of alpha-dystroglycan O-mannosyl glycosylation as well as defects in integrin matrix receptors, the extracellular matrix proteins laminin-alpha(2) and collagen VI, nuclear proteins such as lamin A/C, and a protein of the endoplasmic reticulum, selenoprotein N [9].

The myotonic dystrophies are progressive, degenerative disorders with muscle weakness that share many characteristics with the muscular dystrophies. Myotonia is present both as an electrophysiological and a clinical phenomenon and there are widespread manifestations other than skeletal muscle involvement. An untranslated CTG expansion in the *DMPK* gene on chromosome 19 has been found to cause myotonic dystrophy type 1 (DM1) [10, 11], also known as 'Steinert's disease. Another form is myotonic dystrophy type 2 (DM2), also known as proximal myotonic myopathy, a milder form caused by an untranslated CCTG expansion in the *ZNF9* gene on chromosome 3 [12].

The congenital myopathies (CM) are a heterogeneous group of muscle disorders defined by distinctive morphologic abnormalities in skeletal muscle. In CM there is often a structural defect in a protein situated in the sarcomere or cytoskeleton [13]. In muscle biopsies, intracellular aggregates of various proteins together with other structural abnormalities are seen. The clinical symptoms of muscle weakness and hypotonia are usually present at birth or in early childhood. They are mostly non-progressive and dominantly inherited. The most common congenital myopathy is nemaline myopathy, defined by the presence of rod-shaped structures in muscle fibers, so called nemaline rods.

The etiology behind ion channel disorders is structural defects in one of the ion channels [14]. In Familial periodic paralysis, mutations cause disturbed function of either the Na^+, K^+ or Ca^{2+} channels in the membrane. The diseases are inherited either dominantly or recessively and episodes of periodic paralysis after exercise are common symptoms.

The metabolic myopathies are a heterogeneous group of disorders caused by abnormalities of skeletal muscle energy production, including the breakdown of carbohydrates and fatty acids to generate adenosine triphosphate, predominantly through mitochondrial oxidative phosphorylation [15, 16]. The three main categories are: glycogenoses (disorders of carbohydrate metabolism), lipidoses (fatty acid oxidation defects) and mitochondrial myopathies due to respiratory chain impairment. A variety of clinical symptoms are seen at different ages. In glycogenoses there is a defect in the glycogen synthesis, glycogenolysis or glycolysis. One of the glycogenoses is McArdler, which is caused by mutations in *PYGM*, a gene encoding the muscle-specific phosphorylase [17]. In lipid metabolic disorders, there are defects in intra-mitochondrial β-oxidation enzymes or failure to transport fatty acids into mitochondria secondary to carnitine or carnitine palmitoyltransferase deficiencies [18]. The mitochondrial myopathies are disorders with defects in the final common pathway of energy production, the oxidative phosphorylation [19]. The mitochondrial myopathies can be classified genetically into two major groups: those due to mutations in mitochondrial DNA (mtDNA) and those due to mutations in nuclear DNA (nDNA).

The distal myopathies are disorders defined by onset of muscle weakness in hands or feet, mostly followed by atrophy in muscles of the lower leg, forearm, feet or hands. The clinical course is usually progressive. Today almost 20 different entities of distal myopathies have been genetically determined, including Laing distal myopathy and Tibial muscular dystrophy, and most of the genes encode for protein components of the sarcomere [20].

Other hereditary myopathies are a group of diverse additional diseases that include the myofibrillar myopathies (MFM), characterized by a distinct morphologic pattern of myofibrillar dissolution associated with accumulation of myofibrillar degradation products and ectopic expression of multiple proteins. They have a diverse molecular etiology where all MFM mutations so far identified are in Z-disk associated proteins: desmin, alpha-B-crystallin, myotilin, ZASP, filamin C and Bag3 [21]. The clinical features are more variable, including progressive muscle weakness that often involves both proximal and distal muscles and usually begins in adulthood. Another group of neuromuscular disorders that may be included in the group of other myopathies is the distal arthrogryposis (DA). This is a group of disorders clinically and genetically heterogeneous,

characterized by multiple congenital contractures primarily involving the hands and feet [22]. This may also be a sign of many congenital myopathies as well as of other neurological disorders but in DA syndromes there is usually no major muscle weakness. Most of the mutations in DA identified so far are in genes encoding sarcomeric proteins: tropomyosin, troponin, myosin and myosin binding protein C.

Acquired myopathies

The inflammatory myopathies (IM) [23, 24] are a heterogeneous group of subacute and chronic diseases of skeletal muscle. Common features are moderate to severe muscle weakness and inflammation. They represent the largest group of acquired myopathies and are important because they are potentially treatable. They can be divided in four groups, based on etiology and pathogenesis: 1) idiopathic IM (IIM) which is the largest group, 2) secondary IM that occurs in association with other systemic or connective tissue diseases or with bacterial, viral or parasitic infections, 3) infantile, childhood or congenital forms and 4) miscellaneous forms. Dermatomyositis (DM), Polymyositis (PM) and Inclusion body myositis (IBM) are the three major types of IIM [25]. In DM, muscle capillaries are destroyed by the immune system leading to ischemic damage of muscle fibers. DM has in addition to proximal muscle weakness a typical skin rash [26]. In PM there is a T-lymphocyte mediated attack against muscle cells resulting in progressive muscle weakness [26]. Both DM and PM can be treated successfully with corticosteroids or immunosuppressive drugs [23]. In contrast, IBM is refractory to immunosuppressive treatment. Autoimmune features coexist with degenerative changes in muscle tissue in IBM.

Endocrine myopathies are caused by under- or over- production of hormones, for example thyroid, parathyroid and adrenocortical hormones. If the imbalance of hormone levels is restored, the myopathy is usually reversible [27].

Many different substances are toxic to muscle and can lead to toxic myopathies [28, 29]. For example, alcohol myopathy with severe muscle weakness is found among alcohol abusers. Statins can have myotoxic effects ranging in severity from myalgias to rhabdomyolysis. Critical-illness myopathy is thought to be induced by the usage of neuromuscular blockers in combination with high dose corticosteroids leading to very severe muscle weakness due to massive loss of thick filaments in muscle fibers.

Structure and function of the skeletal muscle

The human body consists of up to 40% of skeletal muscle and 10% of cardiac and smooth muscle, which constitute the three different types of muscle tissue. In contrast to cardiac and smooth muscle, the skeletal muscle is under voluntary control. Skeletal muscle is composed of elongated cylindrical, multinucleated cells, referred to as muscle fibers, formed by the fusion of myoblast cells during development. Each muscle fiber is highly organized with several distinct spatial domains, with the nuclei positioned along the periphery of the fiber, just beneath the plasma membrane (sarcolemma) and the middle packed with the contractile apparatus. The muscle fiber is packed with bundles of filaments that extend the length of the cell. Each bundle, called myofibril, is composed of series of repeated small units, a few micrometers long, so called sarcomeres, which are the smallest unit of contraction. Surrounding the contractile apparatus is a network of sarcoplasmic reticulum, specialized for calcium release and uptake.

Most muscle fibers are only a few centimeters long, much shorter than the length of most muscles. The muscle fiber length is limited by the need of sarcomeres to be activated nearly simultaneously, which in turn is limited by the time it takes for the action potential to travel along the length of the muscle fiber. To achieve an effective mechanical action over larger lengths, groups of muscle fibers, fascicles, are bound together by perimysial connective tissue to form a muscle. Mammalian skeletal muscles are composed of different muscle fiber types that can be divided into three major groups: type 1, 2A and 2B [30]. Most muscles contains a mixture of these different muscle fiber types, which

are recruited to a variable extend when the muscle contract depending on the functional demands. To distinguish between the different fiber types, histochemical methods are used to demonstrate mitochondrial enzymes combined with myosin ATPase activity.

The sarcomere and the contractile apparatus

The sarcomere consists of highly organized protein assemblies and contains bundles of myofilaments, which are longitudinal arrays of actin-containing thin and myosin-containing thick filaments (Fig 1). The thin and thick filaments overlap each other in the dark A-band, whereas the light I-band only contains thin filaments giving the muscle fiber the characteristic striated appearance observed by light microscopy. The thin filaments are anchored in the Z-disk, which constitutes the boundary of individual sarcomeres. Located in the middle of two Z-disks is the M-line. Both the Z-disk and the M-line have structural and signaling role by integrating information related to mechanical strain with signaling pathways, controlling muscle growth and protein turnover. Studies already in the 1950s gave the sliding filament model of muscle contraction where the thick and thin filaments slide along each other without changing in length [31, 32].

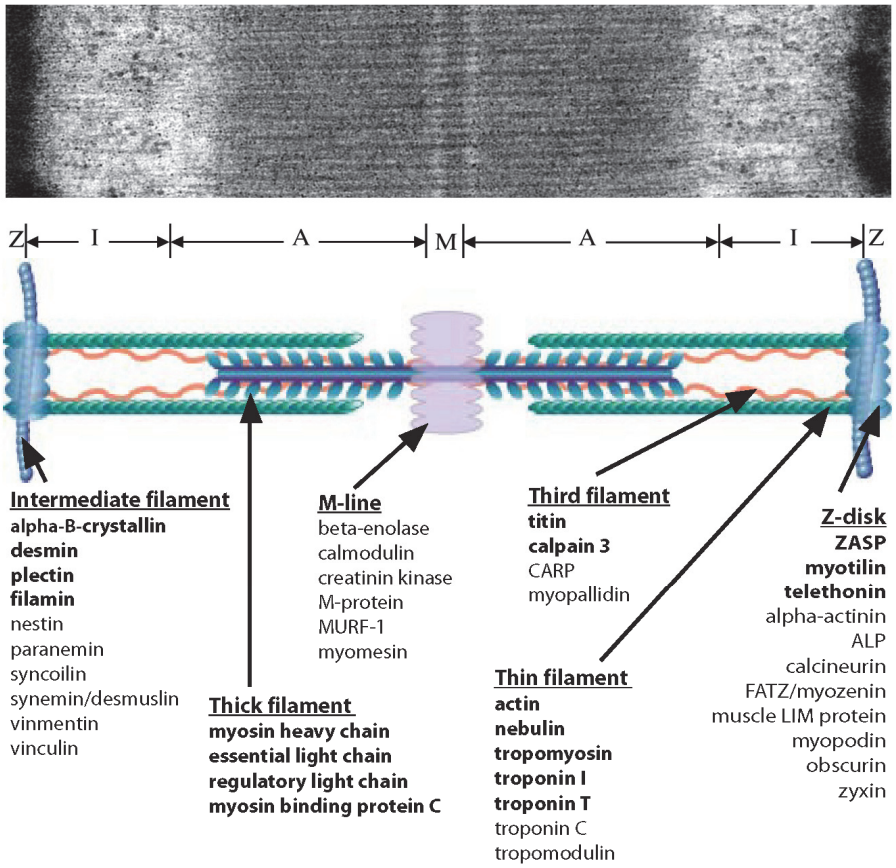

Figure 1. EM picture of a single sarcomere showing I-, A-band, M-line and Z-disk and underneath a schematic diagram showing the major compartments of the sarcomere. Proteins known for each compartment are listed. Proteins that are mutated in skeletal muscle diseases are highlighted in bold. Proteins in plain text have not yet been associated with human diseases.

The thin filament

The thin filaments are mainly composed of actin, which is one of the most abundant proteins in skeletal muscle. The filamentous structure is formed by chains of globular actin molecules (G-actin), polymerized into elongated filaments of double helical strands [33]. Actin monomers (G-actin) have four domains, subdomain 1-4. Each half-helical turn of the actin filament is comprised of seven G-actin molecules, which interact via their subdomains 3 and 4. Subdomain 1 binds to the myosin heads of the thick filaments. In humans, there are six known actin isoforms, each encoded by separate genes whose expression patterns are regulated developmentally and in a tissue-specific manner. Two of the isoforms are striated muscle-specific, skeletal muscle α-actin and cardiac α-actin that are co-expressed to varying degrees in skeletal and cardiac muscle [34].

Except actin, the thin filament also contains tropomyosin (Tm) composed of two α-helical chains, forming a rod-shaped coiled-coil dimer. By overlap of a few residues at the C and N terminals, Tm dimers form a continuous polymer lying in each of the two grooves of F-actin in the thin filament (Fig 2). Tm plays an important role in the regulation of muscle contraction by controlling Ca^{2+} sensitivity and by modulating actin–myosin cross-bridge cycle kinetics in the sarcomere [35]. In adult human skeletal muscle, there are three major tropomyosin isoforms, α-Tm, β-Tm, and γ-Tm, which are encoded by the *TPM1, TPM2*, and *TPM3* genes [36]. *TPM2* and *TPM3* are predominantly expressed in slow fibers, whereas *TPM1* is expressed mainly in fast fibers [35]. In the heart, *TPM1* is the major isoform. *TPM2* also encodes for smooth muscle β-Tm and a form of nonmuscle Tm expressed in fibroblasts. Most nonmuscle Tm isoforms are splicing variants encoded from *TPM1* or *TPM3* [36]. Nonsarcomeric Tm isoforms are present also in muscle [37].

Another component in the thin filament is the globular complex of three proteins: troponin C (TnC) a Ca^{2+} binding protein, troponin I (TnI) an inhibitory protein and troponin T (TnT), a tropomyosin binding protein. The troponins modulate, together with Tm, the interaction of myosin and actin during force generation [38]. A number of isoforms of the Tn subunits are expressed among different fiber types and during development, contributing to the contractile properties of skeletal and cardiac muscles [39].

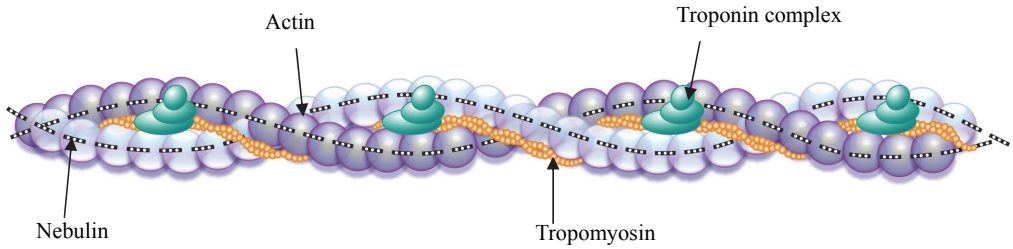

Figure 2. A schematic diagram of the thin filament showing the position of the major protein components. The principal part consists of the double-stranded filamentous actin (F-actin). A single molecule of nebulin stretches the entire length of the thin filament. Tropomyosin dimers form a continuous polymer lying in each of the two grooves of F-actin in the thin filament. The troponin complex consists of troponin C, troponin I and troponin T.

The thick filament

The thick filament consists mainly of myosin, a large molecule with heavy and light chains. Within the A-band, myosin interacts with the thin filament. Muscle myosin exists as a hexameric protein composed of two identical myosin heavy chain (MyHC) subunits and two pairs of non-identical light chain (MyLC) subunits [40, 41]. The head domain of MyHC forms the catalytic motor domain that interacts with actin upon hydrolysis of ATP, where it undergoes a large angular rotation and a displacement. ADP is then dissociated and the actin-myosin complex is returned to the relaxed state. There are three MyHC isoforms in human skeletal muscle, which are associated with the three different fiber types (type 1, 2A and 2B) [42, 43]. Except myosin, there are also other proteins associated with the thick filament implicated in assembly and regulation of muscle contraction such as myosin-binding proteins (MyBP) C and H and adenosine monophosphate deaminase (AMP-deaminase) [44].

Titin -the third filament system

Titin, also known as connectine, is the largest protein identified today in humans, containing 363 exons and encoding 38,138 amino acid residues (4,2 MDa) [45]. The titin molecule spans half of the sarcomere; its N-terminus is part of the Z-disk, it spans the I- and A-bands and its C-terminus is an integral component of the M-line, forming a continuous filament system in myofibrils [46]. In the A band, titin is an integral component of the thick filament where it interacts with the thick filament components MyBP-C, AMP-deaminase and the tail region of myosin [47, 48]. The structural components of the I-band titin have specific elastic properties making titin to function as a molecular spring. In the C-terminus, overlapping the M-line, titin contains a Ser/Thr kinase domain, proposed to be involved in signaling pathways [45]. Many splice isoforms are known, mostly located in the I-band titin, correlating with the structural and elastic properties of the different muscle types.

Nebulin – the fourth filament system

Another giant protein, nebulin (600-900 kDa), forms the fourth filament system in skeletal muscle and is sometimes referred to as a component of the thin filament. The C-terminal is partially inserted in the Z-disk and the N-terminal extends to the "pointed" end of thin filaments [49, 50]. Nebulin is proposed to act as a molecular ruler for specifying the precise lengths of the thin filaments, assembling early during myofibrillogenesis and before actin filaments attain their mature lengths and organization [51]. It is also proposed that nebulin has additional and more complex functions in the sarcomere such as regulating the actin-myosin interaction consistent with studies confirming multiple binding sites to the thin filament, analogous to tropomyosin [52].

Z-disk

The Z-disk is the boundary of individual sarcomeres and constitutes the anchoring site for thin, titin and nebulin filaments. The mechanical force produced by the actin-myosin interaction when the muscle contracts requires a suitable structure and the Z-disk has a central role being the main anchoring point of this molecular machinery. It is now known that the Z-disk is a complex protein network with a high frequency of protein-protein interactions [53]. Many of the Z-disk associated proteins have dynamic distributions in the muscle and may shuttle between the Z-disk and other subcellular locations, for example the nucleus, to transmit signals. One major component in the Z-disk is α-actinin, a member of the spectrin superfamily that cross-links F-actin as antiparallel homodimers in the Z-disk [54]. ZASP (Z-band alternatively spliced PDZ motif protein) is a protein that plays a major role in maintaining Z-disk stability in both skeletal and cardiac muscle. ZASP binds directly to the C-terminal region of α-actinin [55]. Filamin is a protein in the Z-disk that cross-links actin filaments. There are three isoforms, α, β and γ. The γ-filamin isoform is muscle specific and binds to a number of other proteins, including myotilin (myofibrillar protein with titin-like Ig domains) and the δ- and γ-sarcoglycans [56]. It is proposed that filamin can be a critical link between the membrane and the sarcomeric cytoskeleton based on the proteins that it binds to. Myotilin is a Z-

disk protein expressed in skeletal and cardiac muscle and besides γ-filamin, it also interacts with α-actinin and F-actin [57].

M-line

The M-line region is considered to be the anchoring site for the thick filament by cross-linked electron-dense M-bridges [58]. The defined M-line is thought to be the final step in myofibrillar assembly by restriction of the thin filament length and formation of two half-sarcomeres [58]. Today, only a small number of M-line proteins have been identified such as myomesin, muscle-specific RING finger protein-1 (MURF-1) and muscle-specific calpain3.

Intermediate filament

Intermediate filament proteins are thought to be linkers of cytoskeletal networks in the skeletal muscle, although more detailed molecular interactions are still not known and need to be elucidated. They are structurally composed of a central α-helical rod-domain flanked by an N-terminal head and C-terminal tail regions. Desmin is one of the intermediate filament proteins and the component in many regions in the muscle cell (the Z-disk, costameres, the myotendinous junction and intercalated disk) [59]. Other proteins involved in the intermediate filaments include vimentin, nestin, synemin and paranemin.

Myopathies associated with mutations in sarcomere and sarcomere associated proteins

Lately, much effort has been made to elucidate the pathogenesis behind several skeletal muscle diseases that result from mutations in sarcomeric and associated proteins, highlighting their importance in normal muscle structure and function. Many of these diseases affect newborn children, i.e. they are congenital, confirming the fundamental role these proteins have in the contractile apparatus. Mutations in individual proteins can cause multiple different diseases; on the other hand mutations in genes encoding different proteins can cause similar diseases. The clinical and pathological overlaps in these conditions show the complexities of classification and diagnosis. A spectrum of myopathies with different causes and consequences, having the association to sarcomeric and associated proteins in common, are further discussed here.

Congenital myopathies (CM)

The CM are a clinically, pathologically and molecular heterogeneous group of disorders. Four main subclasses can be identified in the group of CM morphologically: myopathies with protein accumulations, with cores, with central nuclei and with fiber size variation. The clinical features are muscle weakness and hypotonia with typically congenital onset and they usually have a non-progressive clinical course. Over the past decade many disease genes associated with CM have been identified, with increasing availability of genetic and prenatal diagnosis [13, 60].

Nemaline myopathy (NM)

NM is defined by the presence of nemaline rods in muscle biopsy specimen (Fig 3). The nemaline rods are expansions and deposits of Z-disk and thin filament material, largely composed of α-actinin and actin [61]. The rods are predominantly found in type 1 fibers, but in a minority of cases they are equally distributed in both type 1 and 2 fibers [62]. The muscle weakness most typically affects proximal muscles, neck flexors and muscles of the torso, but distal muscle involvement is common later in the course of disease. Facial muscle weakness is also common, resulting in long narrow face and high arched palate. The clinical severity ranges from severe cases with neonatal onset and early death to adult-onset cases with only mild muscle weakness. Respiratory and feeding problems are common features, both in the neonatal period and throughout life often requiring nighttime ventilation and tube feeding or gastrostomy insertion. Skeletal involvement includes scoliosis, spinal

rigidity and foot deformities. Cardiomyopathy has only been occasionally described [63, 64]. NM can be divided into six different forms: severe, typical, intermediate, mild, adult onset and other forms, based on the severity of the disease, age at onset, and additional features [65]. Mutations in six different genes are known to cause nemaline myopathy. Five of them encode proteins in the thin filament: skeletal muscle α-actin (*ACTA1*), β-tropomyosin (*TPM2*), α-tropomyosin slow (*TPM3*), nebulin (*NEB*) and troponin T1 (*TNNT1*) and one gene, cofilin (*CFL2)* [66-71], encodes the actin-binding protein muscle cofilin-2. Up to 50% of all NM cases are potentially due to mutations in the nebulin gene and all mutations found today in this gene are recessive, including point mutations or small deletions and rarely, missense mutations [72]. Most mutations in actin are dominant *de novo* mutations but recessive inheritance is also found in some cases. Actin mutations account for 20-25% of all NM cases and more than 50% of the severe cases where many die during the first year of life [66, 73, 74]. Mutations in tropomyosin are a rare cause of NM and onlyone mutation is found in troponin T in an Amish population.

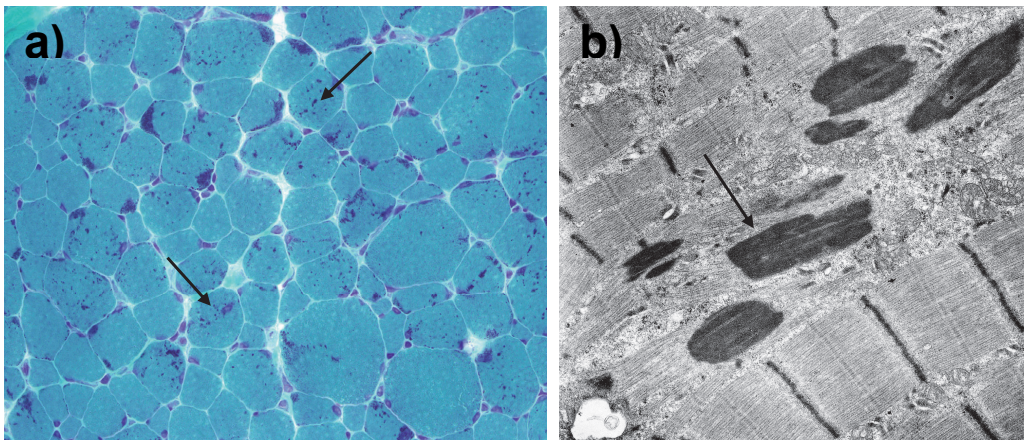

Figure 3. Muscle biopsy sections from a patient with nemaline myopathy. a) Fiber size variation and numerous nemaline rods (arrows) in trichrome staining. b) Electron micrograph showing nemaline rods (arrow).

Cap disease

Cap disease is a congenital myopathy characterized by the presence of cap-like structures (Fig 4), which are well demarcated and peripherally located under the sarcolemma consisting of disarranged myofibrils with enlarged Z-disks and no thick filaments [75, 76.]. The caps show abnormal accumulation of sarcomeric proteins and they show immunoreactivity for many proteins, including actin, α-actinin, tropomyosin, troponin, myotilin and desmin. The clinical features are similar to those of nemaline myopathy, with infantile onset of hypotonia and muscle weakness predominantly involving the proximal muscles, neck flexors, and facial muscles. Respiratory problems are common. Identifying the gene defects behind this myopathy has been one of the aims in this thesis.

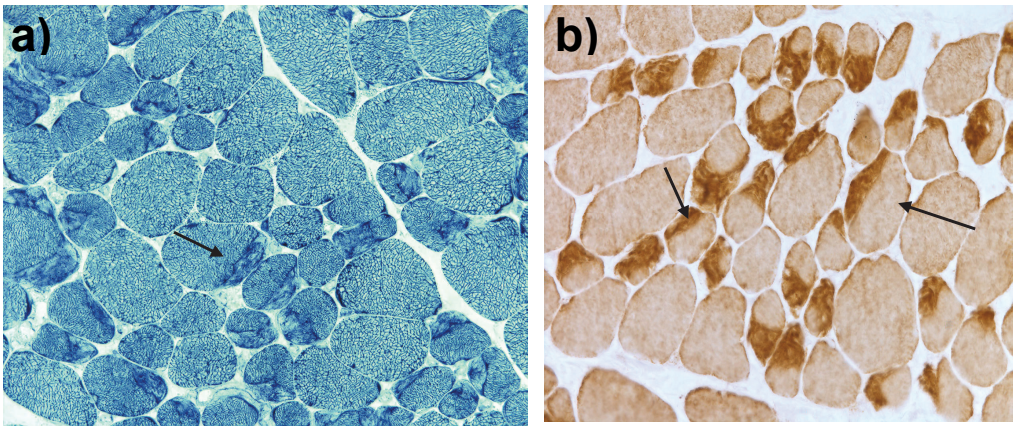

Figure 4. Muscle biopsy sections from a patient with cap disease. a) Subsarcolemmal cap structures in NADH staining. b) The cap structures show intense immunoreactivity for titin (arrow).

Congenital fiber type disproportion (CFTD)

CFTD has, since it was coined in 1973, been characterized by slow type 1 fiber being 12% smaller than the fast type 2 fiber in patients with clinical features of a congenital myopathy [77]. The use of this criteria have given rise to a number of difficulties mainly due to the fact that type 1 fiber hypotrophy is not specific for this disease and is found in a large number of neuromuscular disorders. It has now been proposed that the minimum difference between type 1 and type 2 fiber diameters should be increased from 12% to 25% [78]. The diagnosis should also only be used for cases of idiopathic fiber size disproportion that have clinical features of a congenital myopathy where no other neuromuscular condition can be diagnosed. When this more narrow definition is used, CFTD is an uncommon presentation. The disease-causing genes associated with CFTD today are: skeletal α-actin (*ACTA1*), α-tropomyosin slow (*TPM3*) and selenoprotein N (*SEPN1*) [79-81], which all are known to cause other CM.

Distal myopathies

The distal myopathies are a group of heterogeneous muscle disorders that preferentially affect the distal muscles in early stage of the disease. The clinical course is usually progressive and proximal muscle weakness can be seen later in the course of the disease [82]. The morphologic features vary between the different types of distal myopathies, some of them show dystrophic changes with fiber necrosis and regeneration while other show a variety of myopathic changes. Almost 20 different entities of distal myopathies have been genetically determined and most of the genes encode for protein components of the sarcomere. Laing distal myopathy was linked to chromosome 14q11 in 1995 [83] and ten years later, a mutation in slow beta-myosin gene (*MYH7*) was found [84]. In this disease, the muscle weakness affects the anterior compartment of lower leg and selectively the toe extensor and with a childhood onset as early as four or five years of age. In Welander distal myopathy, a common form in Sweden, there has been a linkage to a locus on chromosome 2p13 [85]. The onset is late, usually after the age of 40, and muscle weakness initially involves the distal extensor muscles of the hands and feet. Tibial muscular dystrophy is another distal myopathy caused by mutations in C-terminal titin, located in the M-line of the sarcomere. It is the most common muscle disease in Finland with late adult-onset of muscle weakness confined mainly to the anterior tibial muscles [86].

Distal arthrogryposis (DA)

Arthrogryposis multiplex congenita (AMC) is a term that is used to describe the presence of multiple joint contractures at birth [87]. The term arthrogryposis has been used as a disease name, but it is now clear that it is not a disease entity but a syndrome, involving many fetal and neonatal disorders of the neuromuscular system. The etiology is varied with the common clinical presentation of decreased fetal movements early in intrauterine life and there is a wide variation in the degree to which muscles and joints are affected. Distal arthrogryposis (DA) is a subgroup of AMC, characterized by congenital contractures in the distal limbs and without obvious neurogenic or myopathic etiology. Ten different clinical forms have been described with DA1 and DA2B as the most common forms [88, 89]. DA1 is the prototypic DA, primarily characterized by camptodactyly and clubfoot, but shoulders and hips may also be affected. The DA1 phenotype is similar to one of the type 2 variants, Freeman-Sheldon syndrome (DA2A), characterized by facial abnormalities with small and pursed mouth, deep-set eyes, hypertelorism and mild micrognathia together with distal joint contractures and short stature. Sheldon-Hall syndrome (DA2B) is defined as a milder variant of DA2A. Mutations in sarcomeric proteins have recently been identified to cause DA: mutations in beta-tropomyosin (*TPM2*) [90, 91], fast troponin T (*TNNT3*) [92], fast troponin I (*TNNI2*) [90, 93], myosin heavy chain (*MYH3*) [94, 95], perinatal myosin heavy chain (*MYH8*) [96] and myosin binding protein C (*MYBPC*) [97].

Myofibrillar myopathies (MFM)

MFM represent a group of muscle disorders clinically and genetically diverse but with a similar morphologic phenotype. The morphologic changes start with myofibrillar disorganization that begins at the Z-disk followed by accumulation of myofibrillar degradation products and ectopic expression of multiple proteins, including desmin, α-B-crystallin, dystrophin, and myotilin. The abnormal fibers contain a mixture of amorphous, granular or hyaline inclusions or deposits that vary in shape and size and are red or dark green in trichrome stained sections. Some of the inclusions may have the appearance of cytoplasmic bodies, which in EM usually are surrounded by a halo of radially arranged thin filaments. The clinical features are variable but include muscle weakness that begins in adult life and evolves slowly. The muscle weakness frequently involves distal muscles but limb-girdle and scapuloperoneal distribution is also common [98]. Cardiomyopathy and peripheral neuropathy are sometimes present and common symptoms are paresthesias, muscle wasting, stiffness, aching or cramps of skeletal muscle, and dyspnea. All mutations found in MFM patients today are in Z-disk related proteins: desmin (*DES*), α-B-crystallin (*CRYAB*), myotilin (*MYOT*), Z-band alternatively spliced PDZ motif protein (*ZASP*), filamin C (*FLNC*) and Bag3 [21, 99, 100].

Hereditary myopathy with early respiratory failure

The first cases of this myopathy were described in seven Swedish families by Edström et al. [101] in 1990. They all exhibited an unusual, adult-onset myopathy with clinical features of proximal muscle weakness in the upper and lower extremities and an early involvement of respiratory and neck flexor muscles. In some cases, muscle weakness was also seen in the ankle dorsiflexors. Muscle biopsy findings revealed myofibrillar changes that included opaque, eosinophilic plaques that stained red or dark green in trichrome staining (Fig 5). These plaques were highly fluorescent when labeled with phalloidin-rhodamine, a specific marker for actin. The ultrastructural myofibrillar alterations indicated early involvement of Z-disks. The same clinical and morphological features have also been described in some other sporadic cases and families [102-104]. Two of the families described by Edström et al. showed linkage to 2q31 [105] and sequence analyses in these two families demonstrated a heterozygous mutation in the titin kinase domain of the titin (*TTN)* gene [106]. The family described by Chapon et al. [102] have demonstrated linkage to 2q21[107] and in the family described by Chinnery et al. [103] linkage to both 2q31 and 2q21 have been excluded, indicating that this type of myopathy is a genetically heterogeneous disorder.

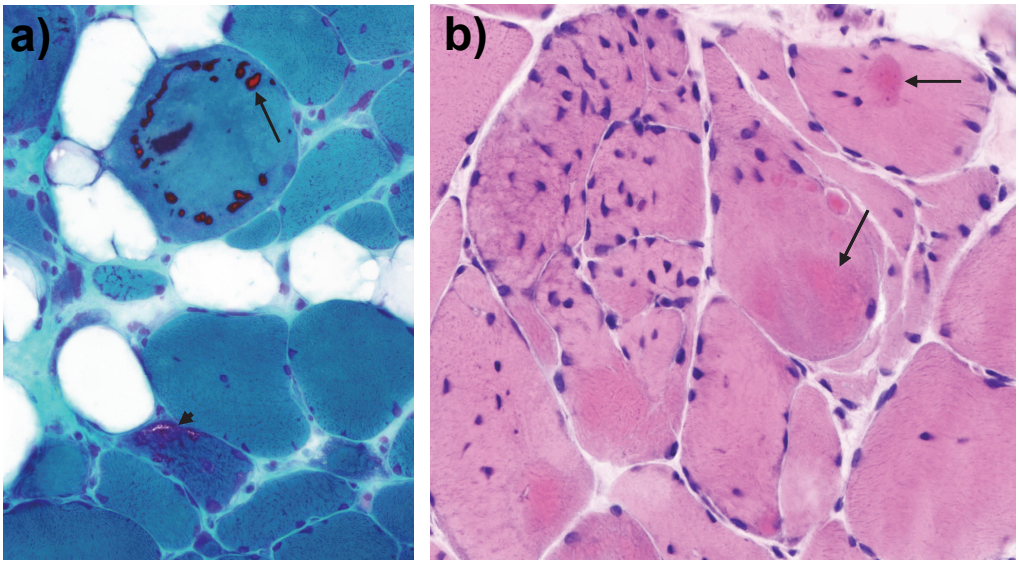

Figure 5. Muscle biopsy sections from a patient with myofibrillar alterations. a) Trichrome stained sections demonstrate red and purple deposits (arrow) and rimmed vacuoles (arrowhead). b) The deposits are partly eosinophilic (arrow) and there are numerous internalized nuclei (Hematoxylin-eosin).

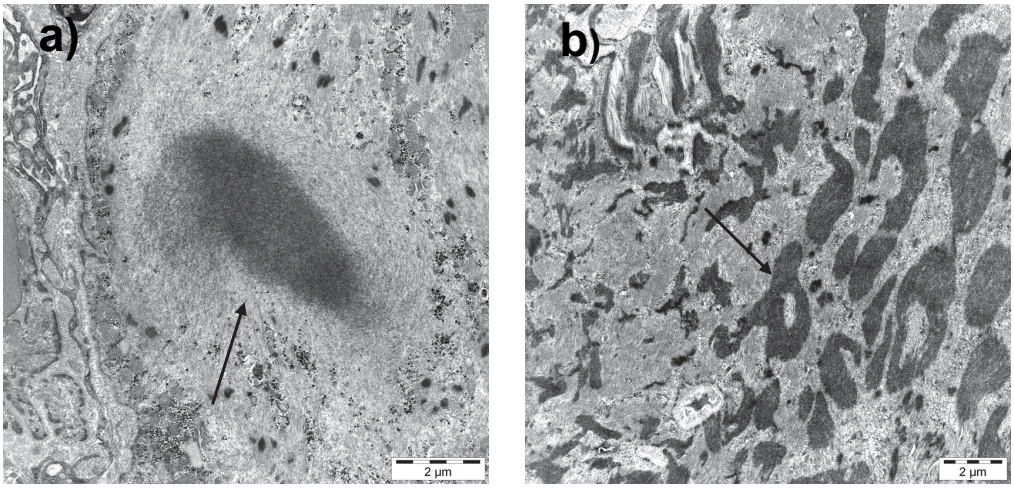

Figure 6. Electron micrograph from the same patient as in fig 5. a) A globular deposit of dense material surrounded by fibrillar material (arrow). b) Loss of sarcomere structure with deposits of Z-disk material (arrow).

AIMS OF THE STUDY

To identify the gene defects associated with nemaline myopathy in two patients and investigate the morphological changes and disease course over time.

To identify the gene defect and to study the clinical and morphological expression in a family (mother and daughter) with a congenital myopathy characterized by nemaline rods and cap structures in muscle biopsy specimens.

To identify the gene defects associated with cap disease and describe the clinical and morphological changes in skeletal muscle in this disease based on the study of four unrelated cases.

To identify the gene defect and characterize the clinical manifestation and morphological expression in two apparently unrelated families with an adult-onset familial myopathy with characteristic morphological changes in skeletal muscle associated with early respiratory failure.

MATERIAL AND METHODS

Patients and clinical investigations *(Paper I-IV)*
The patients had verified muscle weakness and were referred to us for further investigation and diagnosis by physicians at different hospitals. All patients were examined clinically by us or by physicians in their homeland (patient 2 and 3 in paper III and the patient in paper IV).

Patients and clinical investigations *(Paper V)*
Five patients from two Swedish families with clinical signs of muscle weakness were referred to us for further investigation and diagnosis. Two additional affected patients were identified by exploration of the family tree. All patients were examined clinically by us. Muscle strength was evaluated according to Medical Research Council scale (MRC) [108]. Routine investigations of the patients included serum levels of creatine kinase (CK), 12-lead electrocardiogram, echocardiogram and pulmonary function tests. Electromyography was performed in one patient and magnetic resonance imaging (MRI) of muscles in lower extremities in two patients. Muscle biopsy was performed in six patients, and in four of these a second biopsy was performed after seven to ten years.

Controls *(Paper I-IV)*
DNA extracted from blood leukocytes from blood donors and healthy individuals of families with myopathies served as controls for analyses of mutations.

Muscle biopsy *(Paper I-V)*
Skeletal muscle biopsy specimens were obtained by open biopsy of the deltoid, vastus lateralis of the quadriceps or anterior tibialis muscles. The muscle specimens were immediately frozen in propane chilled by liquid nitrogen and stored at –80°C.

Light microscopical morphology and enzyme histochemistry *(Paper I-V)*
Cryostat sections of the fresh frozen muscle biopsy specimens were stained with hematoxylin-eosin, Gomori trichrome, sudan black and PAS. Cryostat sections were also used for enzyme histochemical analyses of myofibrillar ATPase at different pH, NADH-tetrazolium reductase, succinate dehydrogenase and cytochrome c oxidase using standard techniques [109].

Immunohistochemistry *(Paper II, IV, V)*
Immunohistochemical analyses were performed on cryostat sections, which were incubated with the following primary antibodies: Paper II: tropomyosin (Sigma), actin (Dako), α-actinin (Sigma), myotilin (Novocastra), desmin (Dako). Paper IV: tropomyosin. Paper V: NCAM (CD56; Becton Dickinson), desmin, dystrophin (Novocastra), titin (Biocytex), α-B-crystallin (Novocastra), myotilin (Novocastra). EnVision Flex (Dako) was used to visualize immunoreactive matrial. Analysis of actin in Paper V was performed by incubation of sections with phalloidin-rhodamine and fluorescence microscopy.

Electron microscopy *(Paper I-V)*
Samples were fixed in buffered glutaraldehyd and postfixed in OsO_4. Embedding in resin was performed and ultra-thin sections were contrasted with lead citrate and uranyl acetate.

DNA analysis *(Paper I-V)*
Genomic DNA was extracted from frozen skeletal muscle or peripheral blood using DNA Extraction Kit (Qiagen, Hilden, Germany).

DNA sequencing and mutation screening *(Papers I-IV)*
We performed mutation analysis of *ACTA1* in Paper I, *ACTA1, TNNI1, TNNT1, TNNC1, TPM2, TPM3* in Paper II, *ACTA1, MYH1, MYH2, MYH7, TPM2, TPM3* in Paper III and *TPM2, TPM3* in Paper IV. Genomic DNA was amplified by polymerase chain reaction (PCR) performed in a master mixture (ReddyMix PCR Master Mix; Abgene, Epsom, UK) after addition of 20 pmol of each primer and 2 µl DNA. Both forward and reverse strands were sequenced with a Big Dye Terminator DNA sequencing kit using a 377 DNA sequencer (Applied Biosystems, Foster City, CA).

Restriction fragment length polymorphism (RFLP) analysis *(Paper I-IV)*
Screening for the identified mutations in DNA (*ACTA1* in Paper I, *TPM2* in Paper II, III and *TPM3* in Paper IV) from available family members and 100 blood donors was performed by RFLP analysis using different restriction enzymes.

Single nucleotide polymorphism (SNP) array analysis *(Paper V)*
The DNA microarray experiments were performed as previously described [110]. Affymetrix 250K SNP arrays (Affymetrix, Santa Clara, CA) were used and data analysis was performed using GDAS (GeneChip® DNA Analysis software) and GTYPE (Affymetrix) for extraction of genotype calls.

Sequence analysis of a region of the M-line region of *TTN (Paper V)*
PCR amplification was performed of a 1262 bp coding region (position 93761-94933 in *TTN* mRNA, accession number NM_133378) of the first exon at the 3' end of the *TTN* gene encoding the kinase domain referred to as Mex1 (M-line exon). PCR analysis and nucleotide sequence determination were performed as in Paper I-IV.

Ethics
The studies were approved by the regional ethics review board in Gothenburg.

Web-addresses
Information about identified mutations in *ACTA1, TPM2* and *TPM3* was obtained from the Human Gene Mutation Database (http://archive.uwcm.ac.uk/uwcm/mg/search/).

BLAST analyses were performed using the NCBI BLAST web site
(Http://www.ncbi.nlm.nih.gov/BLAST/.

GenBank accession numbers

ACTA1:	AF182035
TPM2:	AF209746, NM_213674, NM_003289
TPM3:	NM_152263
TNNI1:	J04760
TNNT1:	M19308
TNNC1:	X07897
MYH1:	XM_052590
MYH2:	XM_012618
MYH7:	AJ238393

RESULTS AND DISCUSSION

Paper I

Follow-up of nemaline myopathy in two patients with novel mutations in the skeletal muscle alpha-actin gene (*ACTA1*)

The patients in this study were two unrelated children: a 17-year-old boy and an 11-year-old girl with the diagnosis of nemaline myopathy with no family history of neuromuscular disorder. The boy had pronounced generalized muscle weakness, hypotonia and bilateral ptosis at birth. He failed to achieve motor milestones. He has had swallowing and respiratory failure since early childhood. At time of follow-up at age 17 years, he required 24 h ventilatory support and received his feeding through the gastrostomy. Clinical examination showed severe muscle weakness with hypotonia, moderate joint contractures and scoliosis. He had almost no muscle activity as assessed by the MRC-scale except for a limited ability to rotate his neck and left elbow (grade 2), though he could use his hands to control his electric wheelchair and to use a computer. He lived at home with his parents and attended a public school. The girl had feeding difficulties with poor sucking and mild hypotonia in the neonatal period. She could sit at 5 months of age and walked unsupported at 15 months of age. At 20 months of age, she had generalized hypotonia, facial diplegia, and mild, predominantly proximal muscle weakness. At time of follow-up at age 11 years, clinical examination showed a mild generalized muscle weakness, joint hypermobility and she had frequent patellar dislocations. She walked and moved unhindered, could run more than 100 m and attended normal physical education at school. Respiratory investigations showed a mild reduction in vital capacity and normal nocturnal ventilation.

In the boy, the morphological examination revealed marked type 1 fiber predominance, hypoplastic muscle fibers and multiple nemaline rods in virtually every muscle fiber at the age of 5 months. In the girl, a muscle biopsy performed at 18 months of age showed type 1 fiber predominance, variability of muscle fiber size with frequent hypoplastic muscle fibers. Nemaline rods were present in most fibers. A repeat biopsy performed from the same muscle in the boy at age 17 years, showed severe atrophy with replacement of most muscle tissue by fat and connective tissue. The remaining muscle fibers showed type 1 fiber predominance, variability in fiber diameter and disorganization of myofilaments in some fibers. Nemaline rods were only present in a minority of the muscle fibers.

These two patients showed very similar histopathological changes in early childhood. However, the severity of muscle weakness and the disease course differed markedly between the two cases. This discrepancy between similar early muscle pathology and clinical expression has also been observed in previous studies [111]. Our findings observed in the boy, the severe muscle atrophy with replacement of fat and connective tissue, demonstrates the progressive nature of nemaline myopathy that occurs in some cases. The few remaining fibers in general were devoid of nemaline rods, indicating that the most severely affected muscle fibers had disappeared without any effective regeneration. The mechanism by which the fibers die is not clear, but it may be speculated that it involved apoptosis rather than necrosis since there was no evidence of necrosis and s-CK levels were not increased.

We identified two heterozygous mutations not previously described in the skeletal α-actin gene (*ACTA1*), a mutation in exon 6 (p.Gly268Asp) in the boy and a mutation in exon 7 (p.Lys373Glu) in the girl. Both mutations were confirmed by RFLP analyses. The pathogenicity of these mutations is indicated by several factors. There is an extremely low evolutionary sequence variation in skeletal α-actin and the two mutated residues are highly conserved. The p.Gly268Asp mutation caused a change from a non-polar amino acid to a negatively charged residue and the p.Lys373Glu altered a positively charged amino acid to a negatively charged residue, and other mutations in the affected

residues have previously been associated with nemaline myopathy. The mutations were not identified in the healthy parents or in any of 200 control chromosomes providing additional evidence for the pathogenicity and demonstrate the *de novo* dominant nature of the mutations.

Polymerization of globular actin (G-actin) leads to a structural filament (F-actin) in the form of a two-stranded helix. Each half-helical turn of the actin filament is comprised of seven G-actin molecules, which interact via their subdomains 3 and 4. The p.Gly268 is located in subdomain 4 in a hydrophobic core, which is of major importance for the filament structure [112]. The p.Gly268Asp mutation may therefore cause dominant negative effects on muscle structure by perturbed filament assembly and impaired sarcomeric structure. p.Lys373 is located in the C-terminus of actin in subdomain 1, which binds to the myosin head. Because the p.Lys373Glu mutation is located in a region that is important for myosin-actin binding and myosin ATPase activation, it may cause impaired actin-myosin interaction and secondary functional defects of muscle contraction. The missense mutations previously demonstrated in association with nemaline myopathy affecting p.Lys373 and p.Gly268 have caused other types of amino acid substitutions [66, 73, 113]. Two cases with a p.Lys373Gln mutation showed either mild or typical phenotype [73]. Two cases with a p.Gly268Arg mutation have been described both with severe phenotype, while previously reported cases with a p.Gly268Cys mutation showed either mild or intermediate phenotype [73]. Comparing those cases with our cases indicate that the nature of the substituted amino acids is of importance for the phenotypic expression of the disease. However, other genetic or environmental modifying factors are likely to contribute to the phenotypic expression. Mutations in only a few amino acid residues in *ACTA1* have been demonstrated to cause changes to more than one other amino acid as in p.Gly268 and p.Lys373. It is not known whether these residues are especially prone for mutation or have particularly crucial functions in actin. There is however evidence that most actin residues are critical for function as no polymorphic amino acid variants have been identified among hundreds of normal *ACTA1* alleles sequenced [73].

Paper II

Congenital myopathy with nemaline rods and cap structures caused by a mutation in the β - tropomyosin gene (*TPM2*)

The patients in this study were a 66-year-old woman and her 35-year old daughter. The mother had respiratory insufficiency in the neonatal period. Motor milestones were delayed and over the years she had experienced a slowly progressive muscle weakness. Clinical examination at age 57 years showed moderate muscle weakness involving both proximal and distal muscles. She had a myopathic face, micrognathia and lumbar hyperlordosis. She received nocturnal noninvasive ventilation. The daughter had hypotonia and feeding difficulties with poor sucking in the neonatal period. Motor milestones were delayed. At age 2.5 years, she had moderate muscle weakness with predominant involvement of the proximal muscles, neck flexors, and facial muscles. Clinical examination at age 35 years showed muscle weakness in both proximal and distal muscles, myopathic face, bilateral ptosis, micrognathia, and scoliosis. As her mother, she received nocturnal noninvasive ventilation. At this time, she could walk only short distances and was otherwise confined to a wheelchair.

Morphological examination in the mother showed primary myopathy with fiber size variation in the first biopsy at age 32 years. In the daughter the first biopsy at age 2,5 years showed small type 1 fibers. Neither of these biopsies showed any rods or other inclusions. A second biopsy was performed in both patients showing type 1 fiber uniformity and considerable variability of fiber size. The majority of the small muscle fibers showed peripherally located caplike structures, sharply demarcated from the rest of the fiber. In the mother, the caplike structures included numerous nemaline rods. In the daughter, no such rods were found. On electron microscopy, the caps were

composed of disorganized myofibrils and thickened Z bands. Thick filaments appeared to be partly lacking. The caplike structures appeared very similar to what has been described in cap disease [75, 76]. In staining for myofibrillar adenosine triphosphatase (ATPase), the caps showed reduced enzyme activity in both patients. In both patients, the caplike structures displayed strong immunoreactivity for all studied sarcomeric proteins as well as desmin.

Our findings of cap disease and nemaline myopathy in the same family indicate that these diseases are related. We sequenced different genes associated with nemaline myopathy. Mutation analysis of β-tropomyosin (*TPM2*) identified a heterozygous missense mutation in exon 2, c.360G>A, changing the highly conserved and negatively charged glutamate at position 41 to the positively charged lysine in both patients. The mutation was not identified in any of the 3 investigated healthy relatives or in any of 200 control chromosomes.

Tropomyosin (Tm) is localized head-to-tail along the length of the thin filament, providing stability, and is essential for myosin-actin interaction [35]. The binding of calcium to the troponin complex induces the movement of Tm within the thin filament and thereby allowing the binding of myosin to actin [35]. Three major Tm isoforms are present in striated muscle. Beta-Tm (encoded from *TPM2*) is mainly expressed in slow, type 1 muscle fibers. Thus, the identification of a mutation in *TPM2* in our family is compatible with the muscle biopsy finding of structural alterations in slow, type 1 muscle fibers. *TPM2* mutations had so far been described and associated with four different phenotypes: nemaline myopathy, distal arthrogryposis type 1, myopathy without rods combined with distal arthrogryposis type 2B, and a congenital myopathy without rods [68, 90, 91]. This variation indicates that the functional and structural consequences differ between the mutations in *TPM2*. This may be due to interactions with other proteins, which are important for the function of Tm. Our two patients, a mother and daughter with similar clinical but different morphological features suggested that cap disease may be a variant or early stage of nemaline myopathy. Therefore candidate genes in cap disease ought to be found within the genes encoding for sarcomeric proteins, most likely the thin filament or Z-disk and especially those previously associated with nemaline myopathy.

Paper III

New morphologic and genetic findings in cap disease associated with β-tropomyosin (*TPM2*) mutations

Three patients were investigated in this study: two patients from France, a 42-year-old male and an 8-year old girl, and one patient from Sweden, a 6-year-old boy. No history of neuromuscular disorder was found in these cases. The first male patient had no signs of any muscular weakness or hypotonia in the neonatal period but he had difficulties in sitting without support at age 8 months. During puberty, from age 12 to 15 years, he lost the ability to walk longer distances, and from age 15 years, he has been confined to a wheelchair outdoors. Muscle weakness has then been stationary. At age 30 years he had a generalized, symmetric muscle weakness, kyphoscoliosis, winging of the scapulae, and bilateral pterygium colli. Pulmonary vital capacity was reduced. He could still walk short distances at age 42 years. The 6-year-old boy had a normal neonatal period. Hypotonia was noticed at the age of 3 months and he walked unsupported at age 17 months. At age 3 years, clinical examination showed a myopathic face, winging of the scapulae, increased lumbar lordosis, and pes planovalgus. Muscle weakness was most pronounced in the neck and trunk flexors, compared with the neck and trunk extensors, shoulder girdle and hip girdle muscles, and more distal muscles. He walked unhindered but ran with great difficulties. There has been a continuously slow improvement. The third patient, the 8-year-old girl, was hypotonic and had insufficient respiratory function at birth. Motor development was delayed. Nocturnal ventilation was started at age 2 years and at age 5 years she was tracheostomized. At age 8 years she had facial diplegia, bilateral ptosis, limited ocular

movements and weak neck flexor muscles. Strength in the girdle muscles and distal limb muscles were better preserved but she had difficulties walking on her heels. She had lumbar hyperlordosis and a mild thoracic scoliosis, proximal hypermobility of the upper extremities, valgus feet. Mild joint contractures in the hip, finger flexors and in the spine were also seen.

Morphological examination showed cap structures in all three patients. The cap structures were most frequent in the second muscle biopsy in the 42-year-old male patient, not seen in his first biopsy at age 15. In the 6-year-old boy, cap structures could only be found by electron microscopy. The caps were dark in hematoxylin and eosin, modified Gomori trichrome, and NADH-tetrazolium staining, but weakly stained after incubation for myofibrillar ATPase. Electron microscopy showed disorganization of myofibrils, partial loss of thick filaments, and thickened fragments of Z-disks. The muscle biopsies from all patients also showed increased variability in fiber size and near or total uniformity of type 1 fibers. The most striking feature was a coarse-meshed, irregular intermyofibrillar network, myofibrils of irregular size and shape, and jagged Z-disks. Because tropomyosin forms an essential part of the thin filaments, it may be speculated that the abnormalities of myofibrillar size and shape may reflect an abnormal assembly of the thin filaments and that the jagged appearance of Z-disks may be caused by a defective anchorage of the thin filaments in the Z-disks. Because cap structures may be sparse, this can be an important clue to a correct diagnosis.

In this study we tested our hypothesis that mutations in β-Tm (*TPM2*) are a common cause of cap disease. Sequencing of *TPM2* in these patients revealed three different mutations: a heterozygous three-base in-frame deletion (c.384_386delGAA) skipping the highly conserved lysine at position 49 in exon 2 in the male patient, a heterozygous three-base in-frame duplication (c.392_394dupGGG) resulting in the addition of a glycine between the glycine at position 52 and the threonine at position 53 in exon 2 in the boy, and a heterozygous mutation c.845C>G resulting in a conversion of asparagine at position 202 to lysine in the girl. Our results demonstrate that mutation in *TPM2* is a common cause of cap disease, which also has been confirmed by other groups [114, 115].

Several facts provide evidence that the identified mutations in *TPM2* cause the disease. None of the mutations were identified in the healthy parents or siblings and had therefore occurred *de novo*. Loss or insertion of a residue, as in patients 1 and 2, in the highly organized β-tropomyosin molecule, which forms coiled-coil dimers, may be predicted to cause defective assembly or perturbed interaction with other proteins. The β-Tm p.Asn202Lys missense mutation in patient 3 affects a highly conserved residue including all skeletal muscle sarcomeric Tm isoforms and different species, such as *Drosophila* and *Caenorhabditis elegans*.

Tm dimers form a continuous polymer lying in each of the two grooves of F-actin in the thin filament [35]. Troponin T interacts with an extended region including the C-terminal half of the molecule from residue 136 including the overlap region to residue 30 in the N terminal of the following Tm molecule. Only one of the three *TPM2* mutations reported in this study (p.Asn202Lys in patient 3) is located in the putative troponin T binding region of Tm, and therefore a disturbed interaction between troponin T and Tm seems less likely as the major cause of disease. On the other hand, patient 3 was the most severely affected of the three patients, and this might be related to an altered interaction with troponin T. The mutations could possibly disturb the assembly of Tm dimers, which would in most instances be composed of one α-Tm and one β-Tm chain because this is the preferred form when both isoforms are present [35]. An alternative explanation would be that even if the Tm dimers are formed, their interaction with actin is disturbed, which may affect the assembly of thin filaments. The potential actin-binding sites are seven bands of amino acids distributed along the Tm molecule, but the exact interaction sites have not been identified. Reduced binding of Tm to actin was demonstrated for the *TPM3*, α-Tm slow (p.Met8Arg) mutation, which has been associated with nemaline myopathy [116]. It was also demonstrated that the p.Met8Arg mutation perturbs the normal Tm heterodimer formation [117]. Because Tm is a regulatory protein important for muscle contraction, the disease mechanism may involve a functional disturbance. The β-Tm (p.Glu41Lys) mutation described in Paper II was studied by in vitro studies on single muscle fiber preparations in our two

patients and in eight healthy controls [118]. The results showed decreases in speed of contraction at saturated Ca^{2+} concentration and decreases in contraction sensitivity to Ca^{2+} concentration. This suggests that the mutation had a negative effect on contractile function, contributing to the muscle weakness. The β-Tm (p.Arg133Trp) mutation, associated with distal arthrogryposis, showed an impaired regulation of the muscle contraction with the same method [119] and the β-Tm (p.Arg91Gly) mutation, which is also associated with distal arthrogryposis, had a striking effect of increasing myosin ATPase activity as demonstrated by an in vitro assay [120].

Paper IV

TPM3 mutation in one of the original cases of cap disease

The patient in this study was a 38-year-old Polish woman with cap disease, first described by Fidzianska et al. [76] in 2002. She had no family history of neuromuscular disorder. In childhood she had somewhat delayed motor milestones and mild motor difficulties were seen from age 5 years. At age 21 years she fell ill with pneumonia and was tracheostomized due to respiratory failure. She then had generalized muscle atrophy, hypotonia, a myopathic face and kyphoscoliosis. The muscle weakness was mild and she walked unsupported. She had no signs of cardiac involvement. At 38 years of age, she still needed mechanical ventilation at night, her muscle weakness had been stationary and she could still walk independently.

Morphological examination of muscle biopsy specimens at age 18 years showed cap structures in 20-25% of the muscle fibers. A second biopsy was performed three years later that was similar to the previous one in terms of the number of affected fibers and the architecture of caps. The caps appeared dark in NADH-tetrazolium staining and they showed intense immunoreactivity for tropomyosin, actin, α-actinin and desmin. By electron microscopy, the caps demonstrated loosely arranged myofibrils with only a few thick filaments. There was a complete type 1 fiber uniformity consistent with our findings in the three patients in Paper III and earlier described cases with cap disease and *TPM2* mutations. The coarse-meshed, irregular intermyofibrillar network and myofibrils of irregular size and shape, and jagged Z-disks were not found in this patient.

In this study we tested our hypothesis that mutations in cap disease ought to be found within the genes encoding for sarcomeric proteins, most likely the thin filament or Z-disk and preferentially in *TPM2*. No mutation was found in *TPM2,* but sequencing of the α-tropomyosin slow (*TPM3*) gene revealed a heterozygous missense mutation in exon 5 (c.502C>T, p.Arg168Cys), not found in either two healthy parents or in her healthy sibling. This mutation changed the highly conserved, positively charged arginine to the uncharged cysteine. The α-Tm slow is expressed predominantly in slow type 1 fibers. Mutations in *TPM3* have been found to be a rare cause of nemaline myopathy but a more common cause of congenital fiber type disproportion (CFTD) [80]. Missense mutations at position 168, as in our patient, have previously been reported in two unrelated families with nemaline myopathy (p.Arg168His) [121] and also in three unrelated families with CFTD (Arg168Gly, Arg168Cys, and Arg168His) [80] indicating that the p.Arg168 residue is a hot spot for mutations in TPM3. Our patient showed no fiber type disproportion and no nemaline rods. The patients with mutations at position 168 in *TPM3* all share the clinical features of a congenital myopathy, although marked variations are seen according to severity, age at onset, and progression. It is thus clear that missense mutations affecting p.Arg168 in *TPM3* can cause various phenotypes, both morphologically and clinically. The observation that cap disease, like nemaline myopathy, is associated with mutations in *TPM2* as well as in *TPM3* and show similar clinical presentation supports our hypothesis that cap disease is related to nemaline myopathy. All genes encoding components of the sarcomeric thin filament should therefore be considered as candidate genes in patients with cap disease.

Paper V

Familial myopathy with early respiratory failure and sharing of a large haplotype at chromosome 2q31

The patients in this study were seven individuals from two apparently unrelated families, living in the southern part of Sweden. Clinical examination showed muscle weakness in the pelvic girdle, neck flexors and trunk muscles together with prominent calf hypertrophy in all patients. Most of them also had muscle weakness in the knee flexors and ankle dorsiflexors. Examination of lung function showed decreased vital capacity in all patients as a result or respiratory muscle weakness leading to respiratory failure in the disease course. Four out of seven patients had ventilatory support at night. There were no signs of cardiac muscle involvement. Serum creatine kinase (CK) was either normal or slightly elevated, as determined in three patients. Electromyography was performed in one patient and showed myopathic changes.

Magnetic resonance imaging in two patients in one of the families demonstrated selective involvement of specific muscles. The most striking findings in our patients were the difference between the proximal and distal part of the thighs in both patients. While the proximal parts showed almost complete atrophy and severe fatty replacement, the distal parts were nearly normal. The underlying mechanism for these findings needs to be explored.

Morphological examination in these patients showed eosinophilic inclusions in all biopsies that were red or dark green in trichrome staining. Some of them appeared to correlate to cytoplasmic bodies and were fluorescent in phalloidin-rhodamine analysis as a result of their actin content. By electron microscopy, irregular electrondense deposits were found, but they only rarely had the typical structure of cytoplasmic bodies with a halo of radially arranged thin filaments around the deposits. There were very frequent Z-disk alterations with Z-disk streaming and regions with extensive flag-like semi-dense extensions of the Z-disks and large regions with myofibrillar disruption. These features are commonly found in myofibrillar myopathies [21]. In several affected fibers with structural abnormalities there were accumulation of desmin, dystrophin and titin, but no or mild accumulation of myotilin and α-B-crystalline.

Respiratory failure as an early symptom in ambulant patients with inherited primary muscle disease is uncommon but has been described in some sporadic cases and families together with distinct histopathological alterations, such as ultrastructural myofibrillar alterations and cytoplasmic bodies [102-104, 122-124]. Some of these cases have been categorized into the group of "hereditary myopathy with early respiratory failure", first described by Edström et al. [101] in 1990 in sixteen patients from seven Swedish families. In these patients, muscle weakness was found in proximal muscles in the upper and lower extremities, neck flexor muscles, and in some cases also ankle dorsiflexors. The ultrastructural myofibrillar alterations that we observed in this study were similar to those described by Edström et al. and indicate early involvement of Z-disks. It seems, as the group of "hereditary myopathy with early respiratory failure" are genetically heterogeneous. In one family linkage has been found to 2q21 [107] whereas in two of the families that Edström et al. described were linked to 2q31 [105]. Sequence analysis in the two families described by Edström demonstrated a heterozygous mutation (p.Arg279Trp) in the Mex1 exon in the titin kinase domain in the titin gene (*TTN*) [106].

We performed genetic analysis using an in-house statistical model searching for large genomic regions of fitting in an autosomal dominant genetic model. In essence, the method is indirect in that it scores loci where the dominant gene cannot be localized, with the aim of finding a larger region where no such incompatibilities occur, i.e. the inferred disease locus. DNA from five affected patients was subjected to analysis with Affymetrix 250k (250,000 SNPs) SNP arrays and subsequent data analyses. Analysis of the SNP array genotype data using the RFI (regions free of

incompatibilities) model, disclosed a long open (free of incompatibilities) region in chromosome 2q. The data strongly indicated that the depicted region is due to a shared haplotype among the affected patients. The region covers a 29.3 Mb of DNA on chromosome region 2q31 and encompasses 129 known genes including *TTN*. *TTN* was the obvious candidate gene in our patients but sequence analysis of a 1262 bp coding region of the Mex1 exon excluded the p. Arg279Trp mutation and no other mutation was found in the region. *TTN* is still a candidate gene together with other genes of interest in this region.

GENERAL CONCLUSIONS

The inherited myopathies are a diverse group of muscle diseases presenting with common symptoms: muscle weakness and hypotonia. Some of the myopathies are caused by genetic defects in the contractile apparatus of the muscle and are defined by structural or ultrastructural changes on muscle biopsy. Diagnosis and characterization of these disorders are based on clinical history and examination, biochemical and neurophysiological assessment, muscle biopsy, and if available, genetic testing. Today, treatment is focused on symptomatic management and rehabilitation, and monitoring for disease complications. In this thesis some causes of inherited myopathies are described together with their consequences.

In Paper I we investigated two children with nemaline myopathy, included in the group of congenital myopathies. In these patients we identified two *de novo* heterozygous mutations not previously described in the skeletal α-actin gene. The mutations add to the previously reported mutations in *ACTA1* associated with nemaline myopathy. These two patients illustrate the marked variability in the clinical features of nemaline myopathy in spite of similar muscle pathology in early childhood. The severe muscle atrophy with replacement of fat and connective tissue in one of the patients demonstrates the progressive nature of nemaline myopathy that occurs in some cases.

In Paper II we investigated two patients, a mother and daughter, with similar clinical but different morphological features, nemaline myopathy and cap disease. This suggested that cap disease might be a variant or early stage of nemaline myopathy. In these patients a heterozygous missense mutation, Glu41Lys, in the β-tropomyosin gene, *TPM2*, was identified. We concluded that candidate genes in cap disease ought to be found within the genes encoding for sarcomeric proteins, most likely the thin filament or Z-disk proteins and especially those previously associated with nemaline myopathy and that *TPM2* might be a common cause of cap disease.

This hypothesis was confirmed in Paper III were three unrelated patients were investigated. Three *de novo* heterozygous mutations in *TPM2* were identified in these patients: a three–base pair in-frame deletion (p.Lys49del), a three-base pair in-frame duplication (p.Gly52dup), and a missense mutation (p.Asn202Lys). The hypothesis that mutations in *TPM2* are a common cause of cap disease has been supported by other groups [114, 115]. In muscle biopsy specimens from the three patients there were a coarse-meshed and irregular intermyofibrillar network and jagged Z lines. These pathological findings may be clues to correct diagnosis and indicate that the pathogenesis involves defective assembly of myofilaments.

In paper IV we investigated one additional patient with cap disease. In this patient we found a *de novo* heterozygous missense mutation in *TPM3* (p.Arg168Cys). The observation that cap disease, like nemaline myopathy, is associated with mutations in *TPM2* as well as in *TPM3* and show similar clinical presentation supports our concept that cap disease is related to nemaline myopathy and all genes encoding components of the sarcomeric thin filament should be considered as candidate genes in patients with cap disease. This hypothesis has recently been confirmed by the finding of another mutation in *TPM3* and a mutation in *ACTA1* in two patients with cap disease [125, 126].

In Paper V we investigated seven individuals from two apparently unrelated families with a dominantly inherited adult-onset myopathy with early respiratory failure and distinct histopathological features. Similar clinical and histopathological features found in our patients have been described in some other patients and families [101-103] although they seem to be genetically heterogeneous. In two Swedish families linkage to 2q31 has been demonstrated previously [105]. Sequence analyses in these families demonstrated a heterozygous mutation (p.Arg279Trp) in the kinase domain of the titin gene [106]. In another family, linkage to 2q21 was found [107]. Genetic analysis in our two families with array data using SNP markers demonstrated that the affected individuals share a large haplotype on chromosome 2q31 corresponding to a 29.3 Mb region. This region encompasses 129 known genes including *TTN*. *TTN* was the obvious candidate gene in our patients but sequence analysis of a 1262 bp coding region of the Mex1 exon excluded the p.Arg279Trp

mutation and no other mutation was found in this region. *TTN* is still a candidate gene and further studies include the investigation of other portions of the giant *TTN* gene and other genes of interest in this region.

In the group of myopathies associated with mutations in sarcomere and sarcomere associated proteins there are clinical, pathological and genetic overlaps. This is shown by the fact that the same pathological features can be found with mutations in more than one gene and mutations in the same gene can give rise to a variety of pathological features. This highlights the complexities of classification and diagnosis in this group of conditions. In the future, one of the goals is to determine the remaining causative genes behind these diseases and to explore more about the pathogenesis.

One approach to find gene defects is to search among the candidate genes already associated with the diagnosis or among the proteins accumulated in muscle biopsy specimens. This approach was used in this thesis in Paper I-IV. Another approach is to apply the in-house statistical model used in Paper V, searching for large genomic regions shared by the patients. This method can be used as an alternative to conventional linkage analyses in cases of complex and partially truncated family materials, e.g. when there are few affected cases or when older generations in the pedigree are missing. It provides also a possibility to link families and reduce the size of a shared haplotype. In addition this method is very easy to use and the analyses can be run in a common computer software, such as MS Excel.

Although there is no cure today, much can be done to increase the quality of life for the patients with myopathies. For example, physiotherapy can be used to prevent contractures and rigidity secondary to muscle weakness. Antibiotics can be used to treat chest infections that are common secondary to muscle weakness in the respiratory muscles. Non-invasive ventilation can prevent hypercapnia and reduces daytime sleepiness. The use of various technical aids in combination with personal assistance can ease the patients day-to-day activities.

Knowledge of the exact diagnosis is crucial in many ways. It often gives the patient psychological benefits as it is soothing and comforting to know as much as possible about the disease. It gives an opportunity to discuss the prognosis with the patient and it may provide a possibility to tailor follow-up programs, for example in order to detect any signs of cardiac affection or of respiratory impairment, so that therapy can be given prior to symptoms. For patients who want to have children in the future, the possibility of prenatal diagnosis can be very important. This study has deepened the understanding of some inherited myopathies associated with damaged sarcomeres by describing new mutations in causative genes, which in the end could lead to new therapy strategies.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Muskelsjukdomar är en grupp av sjukdomar där det viktigaste symptomet är muskelsvaghet. Sjukdomen kan vara medfödd, debutera tidigt eller debutera senare i livet. Sjukdomen leder i de flesta fall till en livslång funktionsnedsättning vilket medför behov av stora och långvariga insatser från anhöriga, sjukvården och samhället i stort. Det finns omkring 600 olika diagnoser där var och en är mer eller mindre sällsynt. Tillsammans utgör de en grupp med en prevalens (antalet sjuka) på 1/1000 innevånare. Sjukdomarna skiljer sig åt på många olika sätt. De flesta är ärftliga, men har olika ärftlighetsgång. En del är snabbt fortskridande och resulterar i att patienterna avlider inom några år efter symptom debut, medan andra har en mycket långsam eller ingen progress alls. Vissa muskelsjukdomar kan behandlas med mycket god effekt, medan andra är obotliga men där understödjande behandling och rehabiliteringsåtgärder kan ge en förbättrad livskvalitet.

De senaste årens utveckling inom molekylär biologin har medfört att många muskelsjukdomars orsak har kunnat fastställas i form av genetiska förändringar, sk mutationer. Detta har lett till förbättrad diagnostik och prognosbedömning samt möjligheter till fosterdiagnostik. Framstegen har också ökat möjligheterna att kartlägga sjukdomarnas sjukdomsprocess som i vissa fall (recessiva sjukdomar) ofta beror på brist på ett strukturellt protein och I andra fall (dominanta sjukdomar) beror på en abnorm ansamling eller disorganisation av defekta proteiner. Att fortsätta arbetet med att hitta genetiska defekter vid olika muskelsjukdomar tillsammans med ökad kunskap om sjukdomsprocesserna är viktigt för att i framtiden kunna ta fram mer specifika behandlingar vid dessa sjukdomar.

Så mycket som halva kroppsvikten utgörs av muskulatur. Muskeln är uppbyggd av ett stort antal muskelceller, som också kallas för muskelfibrer, som har förmåga till sammandragning (kontraktion) via en invecklad biokemisk process. Denna process involverar bland annat ett stort antal olika proteiner, men framför allt myosin och aktin. Det finns tre typer av muskulatur i vår kropp; skelettmuskulatur, hjärtmuskulatur och glatt muskulatur. Skelettmuskulaturen är den som bygger upp de stora muskler som bland annat finns i armar och ben, och den kallas tvärstrimmig eftersom den ser randig ut när man tittar på den i mikroskop. Skelettmusklerna fäster vid skelettet eller bindväv och kan påverkas av viljan.

Varje muskelfiber innehåller ett stort antal myofibriller som är de trådar som drar samman muskelfibern. Myofibrillerna i skelettmuskulaturen i sin tur består av ett antal segment som kallas sarkomerer. Sarkomererna är den minsta funktionella enheten i en muskelfiber och består av en mängd olika proteiner. Dessa proteiner är sammankopplade i en mycket komplex och exakt struktur vilket är en förutsättning för att muskeln skall kunna utföra sitt arbete. Sarkomererna begränsas i ändarna av Z-band. Utifrån Z-bandet går det trådar av aktin och löst mellan aktintrådarna finns det knippen av myosin som "klättrar" på aktintrådarna i samband med att muskeln kontraherar.

I denna avhandling har vi undersökt ett antal patienter med några olika muskelsjukdomar. Syftet har varit att försöka identifiera bakomliggande genetiska orsaker till patienternas sjukdomar. Samtidigt har en kartläggning gjorts av patienternas symptom där man bland annat tar reda på vilka muskler som är svaga. Alla patienterna har undersökts morfologiskt, dvs genom att ta små provbitar på muskulaturen som man sedan studerar i mikroskop. För att kunna ställa rätt diagnos behöver man ställa samman resultaten från både kroppsundersökning, de morfologiska fynden samt genetiska undersökningar. Tillsammans har detta kunnat leda fram till en säkrare diagnos och prognos för patienterna samt möjlighet till fosterdiagnostik. Utifrån diagnos kan man också följa patienterna på ett bättre och säkrare sätt utifrån eventuella komplikationer.

I det första arbetet identifierade vi två nya mutationer i ett av proteinerna i sarkomererna, skelett muskel aktin, hos två barn. Detta var en studie där vi följde upp barnen vid 17 respektive 11 års ålder. Båda barnen hade haft symptom på muskelvaghet sedan födseln. Vi kunde här påvisa en stor skillnaden mellan sjukdomens svårighetsgrad trots liknande utseende i muskelfibrerna i tidig ålder där det ena barnet var helt beroende av rullstol och var kopplad till ventilator dygnet runt sedan tidig

barndom medan det andra barnet kunde röra sig relativt obehindrat vid 11 års ålder.
Vi kunde också påvisa den svåra muskelnedbrytningen som hade skett hos det ena barnet, där stora delar av muskeln hade ersatts av fett och bindväv. Detta påvisar det progressiva förloppet som sker hos vissa individer med denna muskelsjukdom, nemalin myopati.

I det andra arbetet identifierade vi en ny mutation i ett annat av sarkomerernas proteiner, beta-tropomyosin, hos en mor och dotter. Dessa patienter hade liknande symptom med muskelsvaghet men såg olika ut vid undersökning av muskelfibrerna i mikroskop där de hade fått två olika diagnoser, nemalin myopati och cap disease. Detta var första mutationen som hittats vid cap disease. Vi kunde här dra slutsatsen att dessa sjukdomar är närbesläktade och att de proteiner som troligtvis är förändrade vid cap disease är de som man tidigare sammankopplat med nemalin myopati.

I det tredje och fjärde arbetet fortsatte vi att titta på patienter med cap disease. Vi identifierade 3 nya mutationer till i beta-tropomyosin genen hos 3 av patienterna. Detta bekräftade vår tidigare hypotes om att mutationer i beta-tropomyosin genen kan vara en vanlig orsak till cap disease. Vi fann ett ovanligt grovmaskigt nätverk hos dessa patienter vid undersökningen av muskelfibrerna. Detta speciella mönster kan vara en nyckel till rätt diagnos då man ibland enbart hittar väldigt få cap stukturer vid denna sjukdom som annars behövs för att ställa diagnosen. Vi identifierade också en mutation i en annan gen som kodar för ett annat sarkomer protein, alpha-tropomyosin genen. Man har tidigare hittat mutationer I denna gen hos patienter med nemalin myopati vilket stärker vår tidigare hypotes om att man vid cap disease skall i första hand leta efter mutationer bland de kända generna vid nemalin myopati.

I det femte och sista arbetet undersökte vi sju individer från två olika familjer från södra Sverige med en specifik muskelsjukdom som debuterade sent i livet med muskelsvaghet och andningssvårigheter. I muskelfibrerna såg man ett mycket speciellt utseende med disorganiserade myofibriller. Denna typ av muskelsjukdom har tidigare beskrivits i ett flertal olika svenska familjer. Vi fann att våra två familjer delade ett visst segment i DNA på kromosom 2, en sk haplotyp. Detta talar för att den sjukdomsframkallande genen sitter i detta område som innefattar 129 kända gener. Bland dessa återfinns titin genen, som kodar för ett av proteinerna i sarkomererna. I två av familjerna som tidigare beskrivits har man hittat en mutation i titin genen. Våra patienter hade inte denna specifika mutation men titin är fortfarande en kandidat gen för denna sjukdom.

Sammanfattningsvis har vi identifierat två nya mutationer i skelett aktin genen vid nemalin myopati. Vidare har vi identifierat den första mutationen vid cap disease i beta-tropomyosin genen, samt visat att detta är en vanlig orsak till denna sjukdom. Vi har också funnit att mutationer i en annan gen, alpha-tropomyosin också kan ge upphov till denna sjukdom. Resultaten styrker att cap disease och nemalin myopati är närbesläktade och att man hos patienter med cap disease bör leta efter mutation i de gener som är sammankopplade med nemalin myopati. Vi har också undersökt sjuka individer med en ärftlig muskelsjukdom som debuterar senare i livet med svår andningsproblematik. Våra resultat visar på att den troliga orsaken till denna sjukdom återfinns i en region på kromosom 2. Det fortsatta arbetet kommer att bestå av att titta vidare efter mutationer i denna region.

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