Introduction to Muscle Disease: Pathology and Genetics

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Introduction

There is a very wide variety of disorders that result in muscle weakness, pain, and wasting. The causes of muscle disease range from disruption of the nerve supply and destruction of segments of muscle fibers to interference with the function of individual enzymes or proteins within fibers that characterizes genetic disorders of muscle. Appropriate management and treatment of muscle disease entail close collaboration between clinicians, pathologists, and geneticists, although the balance of involvement of the three groups may vary depending upon the nature of the disease. In adults, muscle diseases are often due to denervation or inflammation as in polymyositis, dermatomyositis, and inclusion body myositis, or to toxic and drug-related myopathies and the effects of aging. However, there is also a growing number of recognized inherited muscle disorders of adult onset. The picture is rather different in children, in whom genetic disorders predominate. During the last two decades, there has been an explosion of research into genetic disorders of muscle and this has changed the way in which clinicians and researchers view muscle disease and challenged the traditional classification of muscle disease.

The aim of this book is to review the whole range of muscle disease using the motor unit and the subcellular components of the muscle fiber to guide the reader through the many different disorders. The concept behind the book is that clinicians, pathologists, and geneticists require an understanding of each other’s disciplines to communicate effectively in the quest for diagnosis and appropriate management and treatment of the patient. In some cases, the diagnosis may be obvious from the clinical presentation and from relatively noninvasive investigations such as electromyography (EMG), magnetic resonance imaging (MRI), and measurements of enzymes such as creatine kinase in the blood. Other cases of muscle disease require muscle biopsy to confirm, pathologically, the presence of denervation, an inflammatory disorder or a reaction to a drug. In a growing number of cases, a muscle biopsy is required to identify the presence of abnormal structures and/or abnormalities in protein expression which, when correlated with clinical features, can aid the identification of a gene defect. This last pathway to diagnosis is often complicated as a defect in one gene may result in a spectrum of phenotypes, or in different phenotypes and pathologies that may overlap with more than one disorder. On the other hand, defects in several different genes may produce similar clinical phenotypes and pathologies. It is the role of this book to set out the pathology and genetics of muscle disease in such a manner that it will guide clinicians, pathologists, and geneticists through the complicated maze of our current understanding of muscle disorders.

Structure of the book

The book is divided into 17 sections, starting with general chapters on clinical features, pathology, and genetics. These are followed by sections related to disorders of nerve supply and genetic disorders of specific subcellular structures in muscle fibers. The book concludes with all-important sections on the inflammatory, toxic, and aging disorders of muscle that often predominate in adults.

Section 1

This section contains three chapters devoted to the general aspects of clinical muscle disease (Chapter 2), to an approach to muscle pathology (Chapter 3) and to the genetics of muscle disorders.
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In subsequent sections, the chapters are laid out in a standard pattern, where feasible, starting with a definition of the disorder and a list of major synonyms. Incidence of the disorder with sex, age, and geographical distribution follow and there is a short account of the clinical features and investigations that are characteristic of the particular disease. A description of the pathology, that may include histopathology, histochemistry, immunohistochemistry, immunoblotting, electron microscopy, and immunological investigations where relevant, is succeeded by an account of the genetics and differential diagnosis where possible. Each chapter ends with a review of animal models and an insight into future perspectives.

Sections 2–17 relate to Figure 1.1. In this diagram, a muscle fiber is depicted with its nerve supply and motor endplate. Components at the surface of the muscle fiber and within the fiber itself are labeled with numbers that refer to the sections of the book dealing with disorders that involve those particular organelles and structures.

Section 2: Neurogenic Muscle Pathology (Chapter 5)

Normal innervation is essential and paramount for the functioning of a muscle fiber. This section covers the major effects on muscle of defects in motor neurons of the spinal cord and of the peripheral nerve axons, many of which are inherited. A subsequent book in the present series will cover these disorders in greater detail. Atrophy of muscle fibers is common to neurogenic disorders of muscle. Early atrophic muscle fibers may be diffusely distributed but later the pathology is characterized by grouping of atrophic fibers. Muscle fibers are reinnervated by surviving axons and by collateral sprouting of axons so that the normal checkerboard pattern of fiber types is disrupted and all fiber types may show grouping.

Section 3: Diseases of Neuromuscular Transmission (Chapters 6 and 7)

Depolarization of muscle fibers that leads to contraction requires not only a fully functional neuron and axon but also a functioning neuromuscular junction. The neuromuscular junction consists of a presynaptic part (the peripheral nervous system) and a postsynaptic part (the muscle fiber) where junctional folds form the subneural apparatus. Diseases of the neuromuscular junction or of neuromuscular transmission are referred to as "myasthenic syndromes" and may affect the presynaptic or postsynaptic compartment. Myasthenic conditions may be acquired, i.e. myasthenia gravis and Eaton–Lambert syndrome, both of autoimmune origin (Chapter 6), or they may be hereditary disorders (so-called congenital myasthenic syndromes) that result from defects in genes encoding proteins localized or enhanced at the neuromuscular junction (Chapter 7). Both disease types affect the threshold of functional postsynaptic acetylcholine receptors and the structure of the junctional folds of the motor endplate.

Section 4: Sarcolemma: Muscular Dystrophies and Related Disorders (Chapters 8–11)

The sarcolemma consists of the outer basement membrane and basal lamina, the plasma membrane (a protein and lipid bilayer), and its associated cytoskeleton. Defects in several proteins of the layers of the sarcolemma cause muscular dystrophies and related disorders (Chapters 8–10). Dystrophin was the first defective protein to be identified in a neuromuscular disorder and this paved the way for the explosion in understanding the molecular basis of muscle diseases. The large dystrophin molecule in the fiber cytoskeleton is connected to a complex of cell membrane proteins that link it to the basal lamina. Defects in this complex and in the extracellular matrix result in a number of disorders, including Duchenne, Becker, limb-girdle and congenital muscular dystrophies, and are associated with varying degrees of destruction and regeneration of muscle fibers, fiber hypertrophy, and fibrosis of the endomysium; all are typical features of a muscular dystrophy but may vary in their severity (Chapters 8 and 9). Other sarcolemmal proteins that are not components of this complex (but may bind to some components) are also associated with other forms of muscular dystrophy (Chapter 10), in which disorders of sarcolemmal ion channels result in disturbances of ionic concentrations that affect muscle contraction and relaxation (Chapter 11).

Section 5: Disorders of Nuclear Proteins and Nuclear Positioning (Chapters 12 and 13)

The nuclear envelope has a specialized complex of proteins that interacts with the nuclear matrix. Defects in these proteins are associated with several phenotypes with overlapping symptoms (Chapter 12). Muscle fibers are multinucleated syncytial cells and nuclei in normal muscle fibers reside beneath the sarcolemma. Nuclei displaced from this normal position are a common feature of pathological muscle which is particularly prominent in some disorders, including those caused by defects in genes encoding proteins of the nuclear envelope. Nuclei in the center of fibers are the pathological hallmark of disorders collectively known as centronuclear myopathies (Chapter 13); interactions between the proteins responsible for these disorders may explain the common pathological feature. Some of the same proteins may also have a role in other disorders (see Chapter 30).

Section 6: Myofibrils: Early- and Late-Onset Disorders (Chapters 14–19)

Proteins of the sarcomere are essential for muscle contraction and defects in several of the proteins result in a variety of disorders of early (congenital myopathies) or late onset. Defects in thin filament proteins are associated with the presence of a particular
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Figure 1.1 The numbers in the diagram refer to the sections in the table and in the book.
structural feature such as nemaline rods (Chapters 14 and 15), and sometimes the accumulation of a protein such as actin (Chapter 14) or myosin (Chapter 16). The congenital myopathies, in particular, highlight the overlap of pathological and genetic defects. Defects in more than one gene may result in a similar pathology, whereas defects in the same gene may result in more than one pathology even in the same muscle sample. In addition, the typical structural feature associated with a defective gene, such as rods, may not be present and then careful clinical assessment and imaging are essential (Chapter 15). Defects in proteins of the Z-disk (such as myotilin, telethonin, ZASP, filamin, BAG3) are also associated with a variable phenotype in disorders collectively termed myofibrillar myopathies. These are often of adult onset and may show similar characteristic pathological features, despite the involvement of different genes (Chapter 17), emphasizing the difficulty of differential diagnosis. Some, however, are of childhood onset and have a severe, rapid progression (Chapters 17 and 19). Titin is a giant protein that stretches from the Z-disk to the M-line of the sarcomere and defects in certain domains are associated with a variety of phenotypes (Chapter 18). Detection of mutations of both titin and nebulin is hampered by their very large number of coding exons (363 and 183 respectively) and by alternative splicing that leads to multiple isoforms. Advances in molecular techniques, such as next-generation sequencing (see Chapter 4) will undoubtedly aid detection of genetic variations in these genes. Application of the technique of laser capture coupled with mass spectrometry led to the identification of FHL1 as the defective protein responsible for the presence of reducing bodies (Chapter 19), and the gene is now known to be associated with a wide spectrum of phenotypes.

Section 7: Disorders Associated with Intermediate Filaments (Chapters 20 and 21)

This is a family of proteins of the cytoskeleton, intermediate in size (10 nm) between thin actin filaments (~7 nm) and microtubules (25 nm). Lamin A/C is an intermediate filament of the nuclear membrane (see Chapter 12). Desmin (Chapter 20) is the muscle-specific intermediate filament that is highly expressed during development of muscle fibers and together with the giant protein plectin (Chapter 21), links the myofibrillar bundles to each other, to other organelles, and to the sarcolemma. Both desmin and plectin are of pathological significance in muscle and aggregation of desmin is an important primary and secondary pathological marker, the hallmark of which is accumulation of granulofilamentous material. Defects in desmin may also cause cardiomyopathy in common with defects in other sarcomeric proteins such as myosin; defects in plectin can also cause a myasthenic condition (see Chapter 9).

Section 8: Mitochondria (Chapter 22)

Mitochondria are abundant in muscle fibers and essential for the production of adenosine triphosphate (ATP). They are located between the myofibrils (Figure 1.1) and in clusters beneath the plasma membrane. Pathology of mitochondria may be expressed by an increase in their number, alterations in size and distribution, abnormal structure of cristae, or by the presence of inclusions. Identification of a mitochondrial defect may require a combination of techniques including histology (presence of ragged red fibers with abundant structurally abnormal mitochondria), enzyme histochemistry (presence of fibers deficient in cytochrome c oxidase), and electron microscopy (visualization of structurally abnormal cristae or inclusions). Pathological studies may reveal no identifiable defect, in which case biochemistry of respiratory chain enzymes and molecular analysis of mitochondrial and/or genomic nucleic acids are very important. Mitochondrial changes can also be associated with aging and as a secondary feature in disorders such as inclusion body myositis (see Chapter 33).

Section 9: Sarcoplasmic Reticulum and T-tubules (Chapter 23)

Sarcoplasmic reticulum and T-tubules play an essential role in excitation and contraction and in the movement of ions, particularly calcium, in response to depolarization of the muscle fiber membrane by a nerve impulse. Defects in the RYR1 gene are associated with a wide spectrum of clinical phenotypes; the histochemical identification of core lesions, devoid of oxidative enzyme activity, led to the definition of a congenital myopathy, central core disease. The identification of molecular defects in the RYR1 gene has broadened the appreciation of clinical and pathological features and the overlap with other disorders. Defects in other genes encoding proteins of the sarcoplasmic reticulum and T-tubules are relatively rare causes of muscle disease but highlight the interaction between organelles within the muscle fiber.

Section 10: Cytoplasmic Proteins (Chapters 24–27)

Cytoplasmic proteins are very diverse. They comprise enzymes such as calpain-3, GNE, chaperone proteins, such as SIL1 and αB-crystallin, Kelch proteins and proteins of autophagy, e.g. LAMP2, VMA21, VCP, and TRIM32. The diseases are accordingly diverse by nature; for example, a muscular dystrophy (LGMD2A) is caused by deficiency of calpain-3 and inclusion body myopathy is due to mutations in the GNE gene. Other disorders in this group include vacuolar myopathies, such as Danon disease and XMEA or sarcotubular myopathy, the Marinesco–Sjögren syndrome, αB-crystallinopathy, and nemaline myopathy type 6. The function and role of several of these proteins are not fully understood and secondary reductions can be a consequence of defects in various proteins with which they interact. For example, calpain-3 may be reduced when dysferlin, caveolin-3 or titin is affected by gene mutations.

Section 11: Metabolic and Storage Disorders (Chapters 28 and 29)

Glycogen and lipid are the essential energy stores of muscle fibers so defects affecting their metabolism have major effects on muscle function. Glycogen is spread across the entire muscle fiber and in normal muscle is seen as granules that can be stained with various
techniques (see Chapter 3). Defects in various parts of the glycolytic pathway result either in storage of glycogen or, more rarely, in abnormal synthesis of glycogen. The major glycogen storage diseases can present in childhood or in adults, and are due to deficient breakdown of glycogen; some of the defective enzymes can be recognized by enzyme histochemistry (Chapter 28). Disorders of lipid metabolism are genetically heterogeneous (Chapter 29) and lipid droplets may accumulate within muscle fibers but this is often not the case, particularly in disorders of adult onset; in these cases biochemical techniques are required to identify the defect.

**Section 12: Muscle Diseases with DNA Expansions (Chapters 30 and 31)**

Most mutations that cause disease affect the coding reading frame but some muscle disorders result from an unstable expansion of a repeat sequence (see Chapter 4). Two forms of myotonic dystrophy (DM1 and DM2) are caused by an increase in the number of repeats on two different genes (Chapter 30), and oculopharyngeal muscular dystrophy by expansion on another gene (Chapter 31). These disorders have several clinical features in common, in particular muscle myotonia, and the pathogenesis is thought to relate to the binding of proteins such as muscleblind that leads to missplicing of several proteins in multiple tissues. Molecular techniques for detecting these disorders are highly reliable so muscle pathology now has a less important role in diagnosis, particularly in DM1, one of the most common inherited disorders of muscle. However, clinical features of DM2 may be less obvious and muscle pathology is then useful.

**Section 13: Facioscapulohumeral Dystrophy (Chapter 32)**

Facioscapulohumeral dystrophy (FSHD) also results from an unusual molecular event which is contraction of D4Z4 repeats at 4q35, and is associated with a specific haplotype. How these missing repeats produce clinical weakness and muscle pathology is unknown. The pathology of FSHD is nonspecific, although myopathic and may be associated with inflammatory infiltrates or with lobulated muscle fibers. There is no specific immunohistochemical marker for FSHD and muscle biopsies are now performed less often.

The last sections of the book (14–17) cover acquired muscle diseases, some of which are amongst the more common muscle disorders.

**Section 14: Inflammatory Myopathies (Chapters 33–36)**

There are several different forms of inflammatory myopathy in which the most characteristic feature is the presence of inflammatory cells (Chapters 33–35). The underlying pathogenesis of these disorders is variable and includes toxins, bacteria, and viruses (Chapter 36) and autoimmune processes. Differential diagnosis is not always straightforward and muscle pathology has a role in identifying the types and distribution of cells present; however, inflammatory cells and typical pathological markers may not be present in all muscle samples.

**Section 15: Toxic Myopathies (Chapter 37)**

There is a wide spectrum of toxic agents that affect muscle and produce clinical symptoms and a variety of pathological changes. An increasing number of such agents are commonly prescribed drugs, such as statins that may result in a necrotizing myopathy, steroids that produce type 2 muscle fiber atrophy, and drugs that affect lysosomal function (such as chloroquine and amiodarone) and result in the storage of lipids within lysosomes. The common denominator in this chapter is the exogenous compounds that damage skeletal muscle.

**Section 16: Aging and Systemic Disease (Chapter 38)**

This chapter addresses the wide variety of factors that affect skeletal muscle during aging and characterizes the resulting myopathy. Neuromuscular disorders associated with cancer, vitamin deficiencies, endocrine disorders, and amyloidosis (most often of the AL or immunological type) are some of the conditions reviewed that emphasize the diverse myopathology associated with aging.

**Section 17: Rare Structural Abnormalities (Chapter 39)**

Many structural defects have been identified in muscle biopsies over the years, some of which have given their name to a disorder; they are discussed in this chapter. A similar chapter was included in the previous edition of this book and the molecular cause of some of the disorders has been elucidated, but it is uncertain if others are genetic entities, as many are isolated cases and only a few rare cases have been identified. The occurrence of familial cases with unusual structures, however, suggests an underlying molecular cause in some. Wider application of techniques such as laser capture and mass spectrometry may lead to a better understanding of these structures.

**Conclusion**

All authors have attempted to give a comprehensive account of the pathology and genetics of muscle disease but new discoveries are published so rapidly that it is not possible to include all the latest advances. The different chapters concentrate on the concepts of the various muscle disorders in the hope that readers will become well equipped to download recent advances from websites such as http://neuromuscular.wustl.edu/ and Online Mendelian Inheritance in Man (OMIM, www.ncbi.nlm.nih.gov/omim) and from searches of the scientific literature.