DEPARTMENT OF HEALTH AND HUMAN SERVICES

Muscular Dystrophy Coordinating Committee

Action Plan for the Muscular Dystrophies

Plan developed by the:

Muscular Dystrophy Coordinating Committee
Scientific Working Group
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Approved by the:

Muscular Dystrophy Coordinating Committee
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MUSCULAR DYSTROPHY COORDINATING COMMITTEE
Action Plan for the Muscular Dystrophies

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Summary Listing of Research Objectives

Note: Research Objectives are grouped so that thematically related objectives appear together within each of the five topics. Their sequential order does not reflect any prioritization of the Research Objectives.

Mechanisms of Muscular Dystrophy

Disease-Specific Pathogenic Mechanisms

- *Research Objective 1:* Define the role that disrupted signal transduction pathways play in the pathogenesis of Duchenne and Becker muscular dystrophies and assess their viability as therapeutic targets (Long Term; Low Risk).

- *Research Objective 2:* Define post-transcriptional mechanisms that lead to muscle (and other tissue) phenotypes in myotonic dystrophy and evaluate the role of DNA repair/replication/recombination mechanisms in microsatellite expansion (Medium Term; Intermediate Risk).

- *Research Objective 3:* Define the molecular pathogenetic mechanisms that lead to facioscapulohumeral muscular dystrophy (Long Term; High Risk).

- *Research Objective 4:* Establish mouse (and cellular) models for facioscapulohumeral muscular dystrophy, specific to emerging candidate genes and/or disease genomics, to understand the epigenetic mechanisms and for the development of novel intervention strategies (Long Term; Intermediate Risk).

- *Research Objective 5:* Define the multiple divergent effects that single mutations in nuclear envelope proteins exert on different tissues in Emery-Dreifuss muscular dystrophy and other laminopathies (Medium Term; High Risk).

- *Research Objective 6:* Determine the pathogenic pathways that are downstream from PABPN1 protein nuclear aggregate formation in oculopharyngeal muscular dystrophy (Medium Term; Intermediate Risk).

General Pathogenic Mechanisms

- *Research Objective 7:* Support continued gene identification and biochemical characterization of rare and understudied forms of muscular dystrophy (Medium Term; Low Risk).

- *Research Objective 8:* Define the roles of inflammation, oxidative stress, and disrupted calcium homeostasis as primary or secondary pathogenic mechanisms in the muscular dystrophies (Medium Term; Low Risk).

- *Research Objective 9:* Characterize defects in post-translational processing and resulting pathogenetic mechanisms in the congenital muscular dystrophies and related disorders (Short Term; Intermediate Risk).

- *Research Objective 10:* Define biochemical mechanisms involved in membrane instability and membrane repair in dystrophic muscle (Long Term; Low Risk).

- *Research Objective 11:* Elucidate apoptotic mechanisms occurring in the muscular dystrophies as potential targets for therapeutic intervention (Long Term; Low Risk).

Infrastructure Needs

- *Research Objective 12:* Establish invertebrate, other vertebrate, and alternative model systems to study pathogenetic mechanisms of gene/RNA/protein defects that cause muscular dystrophies in humans (Long Term; Intermediate Risk).

- *Research Objective 13:* Establish mouse models for dystrophies where none exist, and expand development of mouse models that modify disease phenotype to identify/study disease modifying genes (Long Term; Intermediate Risk).

Diagnosis and Screening of Muscular Dystrophy

Technology for Diagnostic Testing

- *Research Objective 1:* Develop minimally invasive diagnostic techniques for muscular dystrophies where appropriate (Medium Term; Intermediate Risk).

- *Research Objective 2:* Develop definitive gene tests for muscular dystrophies for which genetic testing is not yet available (Short Term; Low Risk to Long Term; Low Risk).

- *Research Objective 3:* Establish mechanisms for muscular dystrophy patients to obtain accurate genetic counseling (Short Term; Low Risk).

- *Research Objective 4:* Establish the specificity and sensitivity of diagnostic tests for the muscular dystrophies (Long Term; Intermediate Risk).

Resources for the Research Community Related to Diagnosis

- *Research Objective 5:* Encourage submission of new mutation and polymorphism data for muscular dystrophy genes to public databases (Short Term; Low Risk).

- *Research Objective 6:* Support disease-specific registries with detailed genetic, structural and functional information regarding phenotypic effects in other organ systems, as well as structural and functional information regarding phenotypic effects in other organ systems, as well as pathological and clinical information. Foster cooperation between registries, neuromuscular research centers, and academic diagnostic centers (Long Term; Intermediate Risk).

- *Research Objectives 7-9:* Optimize utilization of muscle biopsy materials for research by:
    - Developing IRB language to assist investigators with use of archived or prospectively collected diagnostic muscle biopsies (Short Term; Low Risk).
    - Fostering shared use of stored and prospectively collected diagnostic muscle biopsies by the research community (Short Term; Low Risk).
    - Further developing new techniques for evaluation of muscle samples (such as Western blot analysis of very small quantities, proteomics, and laser capture microscopy) and, where appropriate, moving them into the diagnostic algorithm (Medium Term; Intermediate Risk).

Muscle Imaging as a Diagnostic Tool

- *Research Objective 10:* Develop and validate the role of muscle imaging in diagnostic evaluation or as an endpoint measure for clinical trials (Long Term; High Risk).

Epidemiology Studies Based on Genetic Diagnosis

- *Research Objective 11:* Establish current and accurate incidence and prevalence data for genetically confirmed forms of muscular dystrophy (Long Term; Intermediate Risk).

Neonatal Testing for Muscular Dystrophy

- *Research Objective 12:* Develop methods for newborn screening of the muscular dystrophies. Explore the social and ethical issues involved in offering neonatal screening for muscular dystrophy and develop techniques that would make screening practical (Long Term; Intermediate Risk).

Therapy of Muscular Dystrophy

Corticosteroids and Anti-Inflammatory Drugs

- *Research Objective 1:* Optimize the use of prednisone as a treatment for Duchenne muscular dystrophy (Long Term; Low Risk).

- *Research Objective 2:* Determine the mechanism of action of the corticosteroids in muscular dystrophy in order to develop new, potentially more efficacious agents (Medium Term; High Risk).

- *Research Objective 3:* Examine the efficacy of existing anti-inflammatory drugs for treatment of muscular dystrophy (Medium Term; Low Risk).

Growth Factor Modulation

- *Research Objective 4:* Expand insulin-like growth factor 1 (IGF1) preclinical studies and the scope of ongoing clinical trials and understand IGF1's local effects in muscle and other tissues (Short Term; Low Risk).

- *Research Objective 5:* Identify alternative mechanisms of myostatin inhibition and establish their potential as therapeutics through preclinical testing in animal models of various types of muscular dystrophy (Medium Term; Intermediate Risk).

Cell-Based Therapy

- *Research Objective 6:* Achievement of adequate immunosuppression to support myoblast transplant therapy (Medium Term; Low Risk)

- *Research Objective 7:* Define, through basic and preclinical translational studies, the therapeutic potential of alternative muscle progenitor cells (Medium Term; Intermediate Risk).

- *Research Objective 8:* Define, through basic and preclinical translational studies, the therapeutic potential of embryonic stem cells (Medium Term; Low Risk).

Viral Vector Gene Therapy

- *Research Objective 9:* Conduct large animal model testing to determine optimal AAV serotypes for human gene therapy clinical trials (Short Term; Low Risk).

- *Research Objective 10:* Improve the efficiency of gene therapy delivery in the muscular dystrophies, while minimizing the immune response to both gene product and delivery vehicle (Medium Term; Intermediate Risk).

- *Research Objective 11:* Evaluate the clinical endpoints needed for and ethical issues associated with Phase I/II gene therapy and all clinical trials (Medium Term; Intermediate Risk).

Gene Repair

- *Research Objective 12:* Evaluate the safety and efficacy of stop codon readthrough and exon skipping agents through additional translational studies and clinical trials (Long Term; Intermediate Risk).

- *Research Objective 13:* Develop novel agents to improve efficacy of current gene repair strategies or to open new strategies (Medium Term; High Risk).

High-Throughput Screening and Translational Research

- *Research Objective 14:* Identify new strategies to implement translational research projects for muscular dystrophy (Short Term; Low Risk).

- *Research Objective 15:* Expand high-throughput, small molecule screening efforts for promising therapeutic targets and identify novel targets for drug development (Short Term; Low Risk).

Cardiopulmonary Care

- *Research Objectives 16-18:* Improve treatment for cardiopulmonary consequences in muscular dystrophy patients by:
    - Developing guidelines based on evidence and/or current practice standard of care (Short Term; Low Risk).
    - Assessing the value of FDA-approved agents for cardiopulmonary complication prevention (Long Term; Low Risk).
    - Improving management of cardiomyopathy, conduction block, and arrhythmia (Long Term; Intermediate Risk).

Infrastructure Needs

- *Research Objective 19:* Develop the animal models, assays, and tools necessary for preclinical translational research projects that focus upon rapidly moving the accumulated mechanistic knowledge into clinical practice (Medium Term; Intermediate Risk).

- *Research Objective 20:* Improve the availability and distribution network for appropriate mouse models of muscular dystrophy (Short Term; Low RIsk).

- *Research Objective 21:* Ensure the availability and use of large animal models for the later stages of preclinical development (Long Term; Low Risk).

Living with Muscular Dystrophy

Quality of Life Measures in Muscular Dystrophy

- *Research Objective 1:* Identify and evaluate the quality of life and burden of disease measurement tools that are currently available (Short Term; Low Risk).

- *Research Objective 2:* Develop disease-specific quality of life and burden of disease measures where gaps in existing measures are found (Medium Term; Low Risk).

Clinical Endpoints in Natural History Studies and Clinical Trials

- *Research Objective 3:* Determine the sensitivity of clinical endpoints to changes in disease severity (Medium Term; Low Risk).

- *Research Objective 4:* Determine the magnitude of changes in endpoints which are clinically meaningful to patients and family members (Medium Term; Low Risk).

- *Research Objective 5:* Study the interrelationship of clinical endpoints for specific muscular dystrophies (Long Term; Low Risk).

- *Research Objective 6:* Develop standardized data collection approaches nationally using clinically meaningful, readily obtainable parameters; develop a minimum data set for national data gathering efforts (Medium Term; Intermediate Risk).

Consensus Guidelines for the Clinical Management of the Muscular Dystrophies

- *Research Objective 7:* Develop consensus guidelines for clinical management using Duchenne muscular dystrophy as a model (Short Term; Low Risk).

- *Research Objective 8:* Develop consensus guidelines for the clinical management of other muscular dystrophies (Medium Term; Low Risk).

Benefits and Risks of Exercise and Physical Activity

- *Research Objective 9:* Determine the benefits and risks of varied exercise approaches in muscular dystrophies and develop scientifically based recommendations concerning optimal exercise, physical activity, and recreation (Medium Term; Intermediate Risk).

Understanding and Managing the Secondary Consequences of Muscular Dystrophy

- *Research Objective 10:* Assess the prevalence of secondary conditions in muscular dystrophy using existing longitudinal data collection efforts (Short Term; Low Risk).

- *Research Objective 11:* Assess the natural history of secondary conditions in muscular dystrophy using existing longitudinal data collection efforts (Medium Term; Low Risk).

- *Research Objective 12:* Assess the effectiveness of clinical management approaches to prevent and treat secondary conditions using existing multi-center collaborative networks and clinically meaningful outcomes (Medium Term; Intermediate Risk).

- *Research Objective 13:* Define the neuropsychological and neurobehavioral profiles that impact on quality of life and caregiver burden and identify useful interventions (Medium Term; Intermediate Risk).

Patient and Family Education and Social Participation in the Community and Physician Training

- *Research Objective 14:* Establish annual educational conferences for patients and families focused on specific muscular dystrophies (Short Term; Low Risk).

- *Research Objective 15:* Identify strategies to improve patient integration into educational systems (Short-term; Low Risk).

- *Research Objective 16:* Identify strategies to improve vocational outcomes and reduce social isolation (Medium Term; Intermediate Risk).

- *Research Objective 17:* Develop strategies to improve physician effectiveness in communicating with and managing the care of patients with muscular dystrophy (Short Term; Low Risk).

Research Infrastructure Needs for Muscular Dystrophy

Preclinical Research Infrastructure

- *Research Objective 1:* Facilitate research (discovery, validation, and dissemination) of the biochemical pathways involved in muscular dystrophy (Medium Term; Intermediate Risk).

- *Research Objective 2:* Establish standardized endpoints for preclinical trials in both mouse models, and the dog model, and ensure that facilities are available that enable testing of drugs and other therapeutic approaches (Long Term; Low Risk).

- *Research Objective 3:* Create a mechanism to maintain mouse models of muscular dystrophy at approved vendors in a live state, available for easy and rapid importation into academic colonies (Medium Term: Low Risk).

- *Research Objective 4:* Develop optimized models for mechanistic studies of specific muscular dystrophies, including models appropriate for therapeutic development screens (Medium Term; High Risk).

- *Research Objective 5:* Encourage the development of cell-based assays that target aspects of pathogenesis and pathophysiology in the muscular dystrophies, to enable high throughput drug screening (Medium Term; High Risk).

Clinical Research Infrastructure

- *Research Objective 6:* Establish a focus panel for molecular diagnostics of the muscular dystrophies, with the charge of developing consensus standards and approaches for molecular testing, screening, interpretation of results, and genetic counseling (Long Term; Low Risk).

- *Research Objective 7:* Identify, develop, and encourage the use of standardized instruments to measure quality of life, cognitive, and central nervous system function using existing databases and potentially develop new common element databases to extend research capabilities (Medium Term; Intermediate Risk).

- *Research Objective 8:* Monitor, coordinate, and communicate the rehabilitation and educational assessment activities of the various Federal agencies, voluntary, and patient advocacy groups (Long Term; Low Risk).

Communication and Education

- *Research Objective 9:* Design and implement a web site that provides information and links to all existing resources in both the USA and internationally (Short Term; Low Risk).

- *Research Objective 10:* Establish a USA equivalent of the European Neuromuscular Centre's disease focus meetings to link to and communicate with European and other international networks or groups (Short Term; Low Risk).

- *Research Objective 11:* Increase the number and scientific breadth of basic scientists and clinicians involved in translational research in the muscular dystrophies (Long Term; Intermediate Risk).

- *Research Objective 12:* Provide a publicly accessible listing of available training grants and resources so that opportunities for physicians and scientists are transparent (Short Term; Low Risk).

- *Research Objective 13:* Stimulate international collaborations and infrastructure sharing to ensure that opportunities are exploited and resources are used to maximum advantage, particularly in cases of novel opportunity or for the rare and/or understudied muscular dystrophies (Short Term; Intermediate Risk).

INTRODUCTION

The Muscular Dystrophy Coordinating Committee Scientific Working Group was formed in the spring of 2005 and charged with developing recommendations for specific Research Objectives for the muscular dystrophies. The focus of the Muscular Dystrophy Coordinating Committee Scientific Working Group was to identify scientific opportunities in the areas of disease mechanisms, diagnosis and screening, therapy, living with muscular dystrophy, and research infrastructure. This "Action Plan for the Muscular Dystrophies" was designed to specifically identify currently feasible, high-priority Research Objectives that could be used by the Muscular Dystrophy Coordinating Committee and the muscular dystrophy scientific community to coordinate research activities in order to achieve the goal of timely detection, diagnosis, treatment, and prevention of all of the muscular dystrophies.

A. THE MUSCULAR DYSTROPHIES

Muscular dystrophy refers to a group of hereditary, progressive degenerative disorders causing weakness of the skeletal or voluntary muscles. There are many different forms of muscular dystrophy, which differ in their age of onset, penetrance, severity, and pattern of muscles affected. Most types of muscular dystrophy are not simply muscle disorders, but rather multisystem disorders with manifestations in a variety of body systems, including the heart, gastrointestinal system, endocrine glands, skin, eyes, brain, and other organ systems. The major forms of muscular dystrophy include Duchenne/Becker, limb girdle, congenital, facioscapulohumeral, myotonic, oculopharyngeal, distal, and Emery-Dreifuss. Although some forms first become apparent in early childhood, others may not appear until middle age or later.

Duchenne muscular dystrophy, the most common form of muscular dystrophy in children, is an X-linked, severe and progressive degenerative myopathy with early childhood onset. Muscle fiber degeneration and replacement by fibrous tissue and fat occurs in the skeletal muscles and in the heart. Cognitive dysfunction can also occur. It is caused by mutations in the gene encoding dystrophin, resulting in the absence or deficiency of this protein. Becker type muscular dystrophy, which is also caused by mutations in the dystrophin gene, has onset in adolescence or adulthood with a less severe course of progression. An animal model, the mdx mouse, is extensively used to study these disorders.

The limb girdle muscular dystrophies all show a similar distribution of muscle weakness, affecting both upper arms and legs. Many forms of limb girdle muscular dystrophy have been identified, showing different patterns of inheritance: autosomal recessive (designated LGMD1) or autosomal dominant (LGMD2). In an autosomal recessive pattern of inheritance, an individual receives two copies of the defective gene, one from each parent. In an autosomal dominant disease, the disorder can occur in either sex when an individual inherits a single defective gene from either parent. The recessive limb girdle muscular dystrophies are more frequent than the dominant forms, and may be more severe. Limb girdle muscular dystrophy can have a childhood onset, although more often symptoms appear in adolescence or young adulthood. The dominant limb girdle muscular dystrophies usually show adult onset. Some of the recessive forms have been associated with defects in proteins that make up the dystrophin-glycoprotein complex. Mutations in one component of the dystrophin-glycoprotein complex, the sarcoglycans, can lead to the forms of limb girdle muscular dystrophy known as LGMD2C, 2D, 2E, and 2F. Defects in caveolin 3, a protein that associates with the dystrophin-glycoprotein complex, lead to LGMD1C, while mutations in dysferlin, a protein that is thought to interact with

caveolin 3, cause LGMD2B. Mutations in genes not related to the dystrophin-glycoprotein complex are implicated in other forms of limb girdle muscular dystrophy. For example, mutations in the enzymatic protein calpain 3 lead to LGMD2A.

Myotonic dystrophy is the most common form of muscular dystrophy. It is dominantly inherited and characterized by muscle hyperexcitability (myotonia), muscle wasting and weakness, cataracts, hypogonadism, cardiac conduction abnormalities and other developmental and degenerative manifestations frequently including cognitive dysfunction. Penetrance can be variable. Myotonic dystrophy can be caused by mutations in different genes, but the characteristics are quite similar. Type 1 myotonic dystrophy (DM1) is caused by expansion of a CTG triplet repeat in an untranslated region of the dystrophia myotonica protein kinase gene (DMPK) on chromosome 19, while type 2 (DM2) is caused by expansion of a CCTG repeat in the first intron of the zinc finger protein-9 gene (ZNF9) on chromosome 3. Repeat number in the myotonic dystrophies increases in subsequent generations (anticipation). DM1 also has congenital and childhood onset forms; these early appearing forms of the disease differ mechanistically from the adult form only in exhibiting larger CTG repeats that, in turn, trigger earlier appearance of symptoms. Those patients that survive early onset DM1 frequently exhibit morbidity and mortality in the third and fourth decades relating to cardiopulmonary involvement.

Facioscapulohumeral muscular dystrophy is an autosomal dominant progressive degenerative disease that initially affects the muscles of the face (facio), shoulders (scapulo), and upper arms (humeral), followed by the muscles of the feet, pelvic girdle, and abdomen. Affected individuals may also suffer from hearing loss. Onset and progression of the disease is variable and often the weakness is asymmetrical in affected individuals. Life expectancy is typically within normal range, but the disease can lead to severe disability. Nearly all cases are associated with deletions of tandem repeats, termed D4Z4, in a distal region of chromosome 4 (4q35).

The congenital muscular dystrophies are a heterogeneous class of disorders, and include several disorders with a range of symptoms. Muscle degeneration can be mild or severe, and may be restricted to skeletal muscle, or paired with effects on the brain and other organs. Defects in the protein merosin are responsible for about half of the cases in the U.S. Mutations in one of the integrin proteins gives rise to another form of congenital muscular dystrophy. Defects in the proteins called fukutin and fukutin-related protein cause the most common forms of congenital muscular dystrophy found in Japan. All of these proteins are thought to have some relationship to the dystrophin-glycoprotein complex. Some forms of congenital muscular dystrophy, including Fukuyama muscular dystrophy, muscle-eye brain disease, and Walker-Warburg syndrome are due to defective glycosylation of one of the proteins in the dystrophin-glycoprotein complex (alpha-dystroglycan) and show severe brain malformations, such as lissencephaly (a “cobblestone” appearance to part of the brain) and hydrocephalus (an excessive accumulation of fluid in the brain). Other forms, including the merosin-absent form and rigid spine syndrome, do not have major brain malformations associated with the disease. The molecular basis for many forms of congenital muscular dystrophy remains unknown.

Several other forms of muscular dystrophy also occur. Oculopharyngeal muscular dystrophy, which causes weakness in the eye, throat, and facial muscles, followed by pelvic and shoulder muscle weakness, has been attributed to a short triplet repeat expansion in the nuclear polyadenylate binding protein 1 gene (PABPN1), a gene involved in translating the genetic code into functional proteins. Inheritance follows either autosomal dominant or autosomal recessive patterns. Polyalanine tract expansion from a norm of 10 to 12-17 residues causes aggregation of filamentous intranuclear inclusions in skeletal muscle which appear to precipitate the disease. This disease is most common in people of French-Canadian descent or people of Hispanic

descent from certain regions of the Southwest. Miyoshi myopathy, one of the distal muscular dystrophies, causes initial weakness in the calf muscles, and is caused by defects in the protein dysferlin, which is the same gene responsible for LGMD2B, reinforcing the idea that progress against one form of muscular dystrophy should be informative to other forms. There are two forms of Emery-Dreifuss muscular dystrophy—an X-linked and an autosomal dominant form. Emery-Dreifuss muscular dystrophy is characterized by weakness in the shoulder girdle and lower legs, as well as the development of contractures in regions of the body, particularly the elbows, Achilles tendons, and neck. Defects in proteins that make up the nucleus, including emerin, and lamin A/C, are implicated in the disorder.

B. MUSCULAR DYSTROPHY COORDINATING COMMITTEE

The establishment of the Muscular Dystrophy Coordinating Committee (MDCC) was a major provision of the Muscular Dystrophy Community Assistance, Research, and Education Amendments of 2001 (the MD-CARE Act, Public Law 107-84). In accordance with the MD-CARE Act, the Committee is composed of ten members from Government agencies and five members from the public. Government agencies with an interest in muscular dystrophy research and education, including components of the Department of Health and Human Services and the Department of Education, are represented. The Department of Defense is also represented since it received an appropriation for muscular dystrophy research in its FY 2003 Appropriations bill. Dr. Stephen Katz, Director of the National Institute of Arthritis and Neuromuscular and Skin Diseases, chairs the MDCC. A roster of the MDCC is included in this report, immediately following this Introduction.

Purpose of the MDCC

The MD-CARE Act specified that the Muscular Dystrophy Coordinating Committee shall coordinate research activities across the National Institutes of Health and with other Federal health programs and activities relating to the various forms of muscular dystrophy, including Duchenne, myotonic, facioscapulohumeral muscular dystrophy, and other forms of muscular dystrophy. The MD-CARE Act also charged the Muscular Dystrophy Coordinating Committee with the task of developing a plan for conducting and supporting research and education on muscular dystrophy through the National Institutes of Health, and periodically reviewing and revising the plan. The Act specified that the plan shall: (a) provide for a broad range of research and education activities relating to biomedical, epidemiological, psychosocial, and rehabilitative issues, including studies of the impact of such diseases in rural and underserved communities; (b) identify priorities among the programs and activities of the National Institutes of Health regarding such diseases; and (c) reflect input from a broad range of scientists, patients, and advocacy groups.

The MDCC Research and Education Plan for the NIH

Based upon recommendations from the MDCC, a scientific working group was convened in October 2003 to identify research goals in muscular dystrophy. These research goals formed the basis for the Muscular Dystrophy Research and Education Plan for the NIH, which was approved by the MDCC and submitted to Congress in August 2004. This plan provided broad guidelines for the NIH on research priorities for the muscular dystrophies.

At the December 2004 meeting of the MDCC, the Committee agreed that the Muscular Dystrophy Research and Education Plan for the NIH provided broad-based guidance, but that a

second scientific working group should be convened to refine the current plan and to develop and prioritize specific aims for the entire muscular dystrophy research community.

C. MUSCULAR DYSTROPHY COORDINATING COMMITTEE SCIENTIFIC WORKING GROUP

Based upon the MDCC recommendation at its December 2004 meeting, a second Muscular Dystrophy Coordinating Committee Scientific Working Group (MDCC SWG) was appointed. MDCC members were asked to nominate individuals with the appropriate expertise and commitment to serve on the MDCC SWG. Participants were selected to ensure that all of the major types of muscular dystrophy were represented, and that there was sufficient expertise relevant to the topic areas presented in the Research and Education Plan. Twenty-four muscular dystrophy basic and clinical researchers and physicians from outside the NIH participated in the MDCC SWG. Subgroups were formed for each of the five major topics of the existing MDCC Research and Education Plan for the NIH: Mechanisms of Muscular Dystrophy, Diagnosis and Screening of Muscular Dystrophy, Therapy of Muscular Dystrophy, Living with Muscular Dystrophy, and Research Infrastructure Needs for Muscular Dystrophy. The MDCC SWG, in coordination with NIH Staff, developed this "Action Plan for the Muscular Dystrophies."

MDCC SWG Charge

The charge of the MDCC SWG was to:

- Refine and focus the major topics of the MDCC Research and Education Plan for the NIH into specific goals that can be addressed by the NIH and other organizations.
- Prioritize these goals and recommend, where a scientific basis exists, a sequence for addressing them.
- Identify additional obstacles and barriers to the progress of muscular dystrophy research and treatment as well as other outstanding opportunities, noting those that are likely to be addressed through ongoing research and programs, and those that might benefit from additional emphasis.

The MDCC SWG was specifically asked to identify Research Objectives that may help achieve the detection, diagnosis, treatment, and prevention of the muscular dystrophies. The MDCC SWG was to consider all types of muscular dystrophy and identify Research Objectives that were high-priority needs for the field.

MDCC SWG Process

Each of the 24 extramural scientists and physicians appointed to the MDCC SWG was assigned to one of the five subgroups; two co-chairs were appointed in each subgroup. A roster of the MDCC SWG is included in this report, immediately following this Introduction.

The scope of each subgroup was defined by the five major topics in the existing MDCC Research and Education Plan for the NIH. For guidance, the topic subgroup members were provided with copies of both the MDCC SWG charge and the existing MDCC Plan. Through a process of conference calls and electronic communications, each of the five topic subgroups identified: (a) Recent Research Advances that have created new opportunities for progress in

the muscular dystrophies and (b) specific Research Objectives in the area of their subgroup assignment.

Prior to the meeting that was held on August 16-17, 2005, each of the MDCC SWG topic subgroups reached a consensus on the Research Objectives that their subgroup would put forward and prepared a draft report document. At the meeting, each subgroup presented their draft report and used the discussions among the entire MDCC SWG to modify their Research Objectives. The recommendations of each subgroup then were presented and finalized on the second day of the meeting. At the meeting, and in subsequent discussions with the subgroups, the final recommendations and Research Objectives were formatted into a time-risk matrix, indicating the length of time required to achieve the objective and the relative risk in terms of success.

Subsequent to the MDCC SWG, NIH program and policy staff combined the five separate working group documents into a cohesive action plan, and returned the draft chapters and matrices to the MDCC SWG subgroups for final editing and concurrence. The final draft report was submitted to the MDCC and discussed at the November 9, 2005 MDCC meeting. After a 14-day comment period and additional revision, MDCC members voted to approve the Action Plan for the Muscular Dystrophies in December 2005.

Goal for the MDCC SWG Planning Process

The MDCC SWG planning process drew upon the expertise and judgment of leading basic and clinical researchers and physicians in the field of muscular dystrophy. The result is a comprehensive Action Plan for the Muscular Dystrophies that can be used as a guide for progress in the management of these devastating diseases. Implementation of this Action Plan requires the efforts of not only the NIH, but also the involvement and coordination of efforts by all Federal, academic, professional, and patient advocacy group partners committed to the goal of reducing the clinical burden of the muscular dystrophies. Mechanisms will be developed to increase communication and coordination among the Federal agencies and advocacy organizations with a vested interest in the muscular dystrophies and to implement, track, and assess progress in attaining the Research Objectives identified by this Action Plan for the Muscular Dystrophies. The MDCC will be instrumental as a starting point for these activities.

MUSCULAR DYSTROPHY RESEARCH PLANNING PROCESS PARTICIPANTS

A. SCIENTIFIC WORKING GROUP COMPOSITION (* denotes subgroup co-chairs)

Mechanisms of Muscular Dystrophy Subgroup

*Kevin P. Campbell, Ph.D., Investigator, Howard Hughes Medical Institute, and Professor and Head, Department of Physiology and Biophysics, University of Iowa

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Therapy of Muscular Dystrophy Subgroup

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B. OUTSIDE CONSULTANT

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C. MUSCULAR DYSTROPHY COORDINATING COMMITTEE MEMBERSHIP

Stephen I. Katz, M.D., Ph.D., Director, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, and Chair, Muscular Dystrophy Coordinating Committee

Duane Alexander, M.D., Director, National Institute for Child Health and Human Development, National Institutes of Health

Col. Kenneth Bertram, M.D., Director, Congressionally Directed Medical Research Programs, US Army Research and Materiel Command, Department of the Army

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MECHANISMS OF MUSCULAR DYSTROPHY

A. INTRODUCTION

The muscular dystrophies comprise a group of diseases that are genetically, biochemically, and clinically diverse. While they were initially grouped based on pathological similarities, and subdivided by mode of inheritance, age of onset, and distribution of affected muscles, the classification of the dystrophies has been refined over the past two decades by identification of the genetic and biochemical basis of a large number of disorders. A major challenge for research on the mechanisms of muscular dystrophy is to begin to enumerate the various pathogenetic cascades and to identify commonalities and divergences. In fact, the pathologic changes that initially lead to the classification of these different diseases as "muscular dystrophies" are extremely diverse, with few central features that are shared by all. Among the most reliable of those changes would be the end-stage changes of fibrosis, fatty infiltration, variation in fiber size, and evidence of prior degeneration and regeneration (i.e. centrally located nuclei). However, none of these are specific for the dystrophies, as a group they do not reflect early pathogenetic mechanisms, and the particular combination of pathologic findings varies enormously among the different groups lumped together under the general category of "muscular dystrophies." Therefore, it is likely that deeper understandings of pathogenetic mechanisms will result in a further reclassification of these diseases.

Several major conclusions have emerged in terms of disease mechanisms. It seems likely that there will be shared pathogenetic mechanisms among groups of dystrophies, but that there will also be very divergent pathogenetic mechanisms among others. Those muscular dystrophies that are due to mutations in genes encoding dystrophin, sarcoglycans, and laminin may converge very far "upstream" in terms of the mechanisms that lead to muscle cell death, with rounds of degeneration and regeneration, leading ultimately to muscle wasting and progressive weakness. Similar mechanisms may also account for pathologic changes in those congenital muscular dystrophies that are associated with glycosylation defects in alpha-dystroglycan. By contrast, mechanisms leading to the characteristic pathologic changes in myotonic dystrophy, facioscapulohumeral muscular dystrophy, dystrophies associated with mutations in nuclear membrane proteins, and dystrophies related to mutations in calpain, dysferlin, and caveolin 3 are likely to have very different pathogenetic cascades.

Central to the challenge of studying disease mechanisms is the importance of considering each disorder biochemically, rather than just pathologically. This challenge is highlighted by the fact that there is an incomplete understanding of the function of most proteins that are deficient or defective in the dystrophies, either in muscle cells specifically or in cells in general. Dystrophin is clearly necessary for maintaining a stable complex of proteins that link the extracellular matrix with intracellular cytoskeletal elements and signaling proteins, but the fact that even in this most highly penetrant of disorders, muscles that are deficient in dystrophin show highly variable levels of disease activity among different muscle groups, over time, and across species suggests that the pathogenetic mechanisms are complex.

Mutations in nuclear membrane proteins present as diseases of both muscle and other tissues, highlighting the fact that the muscular dystrophies need to be considered as more systemic diseases and that pathogenetic mechanisms may be best understood by studying tissues other than muscle. The effects of mutations that cause facioscapulohumeral muscular dystrophy and myotonic dystrophy clearly do not relate to a single protein deficiency, and the pathogenetic mechanisms in these disorders have required exploration beyond the simple single-gene-mutation/single-protein-deficiency paradigm. In the post-genomic era, the wealth of information

that is becoming available from expression profiling and proteomic studies serves as the starting point for studies of pathogenetic mechanisms of disease.

The ultimate value for mechanistic studies in muscular dystrophy lies in the identification of appropriate targets for therapeutic development. The degree to which the muscular dystrophies have common pathogenic mechanisms will determine the scope of any therapeutic strategy. Comparative studies to understand similarities and differences in the pathogenesis of the different muscular dystrophies may facilitate the development of individual treatments with applicability to a range of types and identify cases where type-specific therapy development is necessary.

B. RECENT RESEARCH ADVANCES

The mechanisms underlying the muscular dystrophies are diverse. The precipitating mechanisms and downstream pathogenesis of those dystrophies that are associated with mutations in genes encoding components of the dystrophin-glycoprotein complex appear to exhibit important commonalities and have testable models that support hypothesis-driven research. Despite this, the precise means by which the loss of one or more dystrophin-glycoprotein complex members translates into muscle fiber death are not yet fully understood. Mechanistic knowledge is considerably less advanced for facioscapulohumeral muscular dystrophy, myotonic dystrophy, Emery-Dreifuss muscular dystrophy, and those limb girdle muscular dystrophies that are not associated with dystrophin-glycoprotein complex defects.

Duchenne/Becker Muscular Dystrophy

Reductions of nitric oxide synthase exacerbates disease pathogenesis. Neuronal nitric oxide synthase (nNOS) is a nitric oxide producing enzyme that is associated with the sarcolemma and a component of the dystrophin-glycoprotein complex. The loss of dystrophin in Duchenne muscular dystrophy results in displacement of nNOS protein from the sarcolemma, reduced nitric oxide release from dystrophic muscle, and decreased circulating nitric oxide levels. There are at least three identified downstream effects of this reduction of NOS that may contribute to the pathogenesis in Duchenne muscular dystrophy:

- The normal release of nitric oxide from muscle fibers promotes local vasodilation to overcome the alpha-adrenergic stimulation that accompanies exercise. The loss of muscle nitric oxide production in muscular dystrophy results in localized ischemia, which may be a contributing factor to disease pathogenesis.

- Nitric oxide serves as an anti-inflammatory agent in muscle. Loss of this function has been shown to contribute to muscle pathogenesis in the mdx mouse model of muscular dystrophy.

- Nitric oxide causes release of hepatocyte growth factor, a protein capable of activating muscle precursor cells (satellite cells). The subsequent loss of nitric oxide production in dystrophinopathy can act through this mechanism to reduce adequacy of repair mechanisms and thereby exacerbate pathogenesis.

Dystrophin-deficient muscle exhibits enhanced susceptibility to oxidative stress. Dystrophic myogenic cells in culture show an increased susceptibility to free radical-mediated stress. In addition, pre-necrotic mdx mouse muscle shows evidence of increased oxidative stress prior to the onset of any other visible dystrophic changes. Collectively, these findings suggest that free radicals released by immune cells, or due to mitochondrial calcium overload in muscle fibers, may contribute to pathogenesis.

Cellular proteases contribute to muscle cell death. Membrane damage in dystrophic muscle allows intracellular enzymes to leak out (detected as elevated serum creatine kinase) and also may lead to abnormally high ion accumulation within muscle fibers. While the issue of elevated free calcium levels in mdx mouse and Duchenne patient muscles is controversial, calcium dependent proteases (calpains) show both elevated concentration and activity in mdx muscles at the peak of muscle necrosis. Calpain concentration is also increased in Duchenne patient muscles. Correspondingly, the suppression of calpains with an endogenous inhibitor (calpastatin) reduces disease severity. The role of calcium overload and calpain activation in the pathogenesis of the other muscular dystrophies is currently unclear.

Recent studies have implicated other proteases in the pathogenesis of muscular dystrophy. Inhibition of serine proteases (using Bowman-Birk Inhibitor) leads to attenuation of dystrophic symptoms. Clinical trials are being planned to test this drug in Duchenne muscular dystrophy patients (National Institute of Neurological Disorders and Stroke).

Dystrophic muscle shows aberrations in signaling pathways. While the predominant theory is that loss of dystrophin results in a mechanical weakening of the sarcolemmal membrane, it is clear that loss of dystrophin has other deleterious effects on muscle cell function and viability. For example, muscle mass can be increased in the mdx mouse through myostatin inhibition, but the increased mass does not translate into an increase in the specific force that the muscle can generate. Therefore, it is likely that dystrophin serves more than just a mechanical/structural function in the cell.

Dystrophin and the dystrophin-glycoprotein complex proteins have several identified signaling functions and loss of the complex in muscular dystrophy has been associated with alterations in multiple signal transduction pathways. Some of these changes may be due to the compensatory upregulation of other proteins with signaling functions, like integrins, and some may be directly related to the loss of dystrophin.

Recent studies have identified specific cell signaling pathways associated with the dystrophin-glycoprotein complex and corresponding signaling aberrations of these pathways in dystrophic muscle, including:

- JNK kinase, a member of the stress-activated MAP kinase family, is associated with the dystrophin-glycoprotein complex. JNK phosphorylates and inactivates NFATc, a protein important in muscle gene transcription. Through its NFATc activity, JNK regulates muscle gene transcription and activity of this kinase is increased in mdx mouse muscle. Deflazacort, a corticosteroid shown to be beneficial in treating Duchenne muscular dystrophy, may over-ride the deleterious effects of JNK activation by increasing NFAT expression.

- ERK kinase and p38 kinase activities are elevated in dystrophin-deficient mouse muscle. But, it is not yet clear if activation of these kinases contributes to muscular dystrophy.

- NFKB is elevated in both pre-necrotic mdx mouse muscle and Duchenne patient biopsies. Elevated NFKB might, in turn, increase the level of pro-inflammatory cytokines in the muscle environment, thus triggering increased inflammation and exacerbating muscle damage. Genetically modified mice with increased NFKB activity show muscle wasting.

- The AKT pathway is activated in response to normal alpha-dystroglycan-laminin interactions. It is hypothesized that loss of AKT signaling through this pathway may decrease cell survival or cell size in the muscular dystrophies.

Inflammation exacerbates the pathogenesis of mdx muscle. Muscles from both Duchenne muscular dystrophy and the mdx mouse have elevated inflammatory cell infiltration and increased inflammatory mediators such as cytokines and chemokines. Reductions in cytokine/chemokine level or in either the activation or concentration of inflammatory cells slow the pathogenesis of muscular dystrophy. In addition, there is a correlation between prednisone treatment and reductions in all inflammatory cell populations, suggesting that the anti-inflammatory actions of prednisone are one reason that this drug is beneficial in Duchenne muscular dystrophy. Anti-inflammatory activity of the steroids is likely due to reductions in the cellular adhesion molecules that are important for immune cell extravasation to the muscle.

Taken together, recent research on perturbations of cell signaling pathways in muscular dystrophy has identified several novel targets for drug design.

Myotonic Dystrophy

Unstable microsatellite expansions cause myotonic dystrophy types 1 and 2. Myotonic dystrophy is the most prevalent form of muscular dystrophy in adults. There are two forms of myotonic dystrophy, DM1 and DM2. While many pathological features are shared, DM1 is the more severe disease, particularly in the congenital form. DM1 is associated with a $(CTG)_n$ expansion in the 3' untranslated region of the *DMPK* gene, while DM2 results from a $(CCTG)_n$ expansion in the first intron of *ZNF9*. The discovery that myotonic dystrophy is caused by structurally related microsatellite expansions in different genes suggests that the repeat expansions themselves are intrinsically toxic.

The pathogenesis of myotonic dystrophy is RNA-mediated. Early mechanistic studies on the pathogenesis of myotonic dystrophy suggested that disease might be mediated by a toxic RNA effect. While it is dominantly inherited, the repeat expansions are located in non-coding regions and myotonic dystrophy mutant allele transcripts accumulate in the nucleus in ribonuclear foci. Conclusive evidence for a toxic RNA effect was provided by the development of HSA^{LR} transgenic mice, which carry a CTG expansion in the 3' untranslated region of the human skeletal actin gene. Only those mice that actively express this transgene develop myotonic dystrophy-associated muscle defects and transgene expression levels directly correlate with the severity of muscle pathology.

RNA splicing mis-regulation is a downstream pathogenic event in myotonic dystrophy. One of the pathways that is adversely affected by expression of toxic DM1 and DM2 RNAs is RNA alternative splicing. During postnatal development, fetal exons must be excluded from mRNAs in order to generate adult isoforms; this regulation is controlled by two sets of antagonistic splicing factors, the CELF and MBNL protein families. Expression of DM1 and DM2 mutant transcripts results in the sequestration of the MBNL proteins which normally promote adult splicing patterns. Thus, myotonic dystrophy most likely is an RNA splicing disease in which fetal isoforms are retained in the adult.

Development of myotonic dystrophy mouse models. The future development of therapies for myotonic dystrophy will be facilitated by the generation of disease models which faithfully recapitulate characteristic features of the human disease phenotype. Several new mouse lines (*Mbnl1* knockout and CUGBP1 transgenic) faithfully model many of the multi-systemic defects associated with human myotonic dystrophy.

Role of DNA mismatch repair genes and genetic recombination in the generation of microsatellite expansions. Somatic mosaicism of the myotonic dystrophy DNA repeats can be recapitulated in both mouse and cell culture models. The mismatch repair genes *Msh2*, *Msh3*,

and *Pms2* are required to generate these DNA expansions. Recent work also indicates that the DM2 repeats are unusually active recombination hotspots that are prone to expansion.

Congenital myotonic dystrophy. Early appearing myotonic dystrophy is seen only for DM1. The molecular basis for early onset lies in the finding that inherited unstable DNA sequences can become larger in each subsequent generation. Congenital and childhood-onset DM1 correlates with CTG repeat size with not other apparent mechanistic differences from the adult disorder. Recognition of the childhood-onset form is important because of associated conduction system abnormalities.

Facioscapulohumeral Muscular Dystrophy

Most cases of facioscapulohumeral muscular dystrophy are associated with the loss of subtelomeric DNA repeat units (D4Z4) from chromosome 4q. In contrast to most monogenic disorders, in which the genetic lesion typically affects the structure or function of a specific protein, there is evidence that facioscapulohumeral muscular dystrophy is associated with a complex epigenetic mechanism involving the contraction of a subtelomeric macrosatellite repeat. In 95% of patients, the D4Z4 repeat is contracted to an array of 1 to 10 units, while unaffected subjects have up to 100 units. The D4Z4 repeat contains the DUX4 gene, which encodes a double homeobox-containing protein. Some familial cases have been reported that do not link to 4qter, but a second locus for facioscapulohumeral muscular dystrophy has yet to be identified. The pathogenic mechanisms underlying muscle weakness and degeneration remain unresolved.

The potential role of the tandem repeat reduction in modulation of transcription of nearby genes has been the subject of intensive investigation. A putative repressor complex has been identified that binds to D4Z4. In muscle of patients with facioscapulohumeral muscular dystrophy, a distance-dependent transcriptional upregulation was found for three genes, FRG2, FRG1, and ANT1, located near the repeat units. However, in independent follow-up studies, the upregulation of these genes could not be confirmed. Hence, considerable controversy exists over whether the loss of D4Z4 repeat units in facioscapulohumeral muscular dystrophy leads to loss of repression of genes near the D4Z4 locus.

Molecular models for facioscapulohumeral muscular dystrophy. Several models have been proposed by which the contraction of the D4Z4 repeat leads to changes in gene expression and the multi-system pathologies associated with this disease. These models include altered gene expression as a consequence of either: (a) the structure of D4Z4 repeats and their interaction with local chromatin or the nuclear lamina, (b) changes in D4Z4 methylation, or (c) expression and activity of DUX4. Critical data pertaining to these or other models may be gained by identifying the mutations outside of the 4q region that are associated with a small percentage of facioscapulohumeral muscular dystrophy patients. There is a significant need for model systems that will facilitate testing of these hypotheses and for a better understanding of the pathophysiology of this disease.

Laminopathies

Mutations in lamins A and C cause muscular dystrophy and other diseases. A relatively recent revolution occurred in the field of muscle pathophysiology when mutations in the nuclear envelope proteins, emerin and lamins A and C, were shown to cause muscular dystrophy (Emery-Dreifuss muscular dystrophy and limb girdle muscular dystrophy type 1B) and dilated cardiomyopathy. This set the stage for analyzing nuclear protein involvement in other hereditary diseases, and since 1999, nuclear lamin A/C mutations have been shown to have tissue/organ system consequences beyond the striated muscle disorder. These diseases include

lipodystrophy syndromes, peripheral neuropathy (Charcot Marie Tooth type 2) and syndromes with features of premature aging.

Mutations in the lamins A and C cause mechanical problems with the nucleus and abnormal cellular responses to stress. Several studies have shown that nuclei containing the lamin A and C mutations associated with muscular dystrophy and other diseases have various abnormalities in architecture, protein dynamics and response to heat and mechanical stress. These studies suggest, but do not yet prove, that susceptibility to mechanical stress-induced injury may be part of the pathophysiological mechanism of how mutations in lamins A and C cause muscular dystrophy.

Nuclear lamins may be involved in transcription control. Several studies have suggested that nuclear lamins A and C are important in processes including DNA replication, cell cycle regulation, and transcriptional control. However, none of these studies thus far have uncovered mechanistic details. Nonetheless, the results of such studies have led to the hypothesis that mutations in lamins A and C and other nuclear envelope proteins may cause disease by inducing tissue-specific abnormalities in gene expression, possibly activating or deactivating important cell signal transduction pathways.

Lamin contributions to nuclear envelope protein structure. The complete three-dimensional structures of the nuclear lamins A and C, or any other intermediate filament protein, are not known. In the past few years, some advances have been made in determining the three-dimensional structures of various domains of the nuclear lamins and other nuclear envelope proteins. These studies have provided some clues about possible functions and how mutations may cause different human diseases.

Mouse models of mutations in nuclear envelope proteins. Several mouse models, specifically lamin A/C knockout and knock-in mice, have been developed for human muscular dystrophies and other diseases caused by lamin A/C mutations. While these models do not precisely mimic the corresponding human disorders, they have been extremely useful to investigators in studying pathogenic mechanisms of the laminopathies.

Congenital Muscular Dystrophies

Glycosylation defects in limb girdle and congenital muscular dystrophies. The post-translational modification of sarcolemmal proteins represents a new focus for mechanistic studies of muscular dystrophy. Several genes that encode either known or putative glycosylation enzymes have been causally associated with various types of muscular dystrophy. The pathogenic mechanisms of these diseases converge into abnormal glycosylation. In particular, mutations in glycosyltransferase enzymes cause a novel group of human muscular dystrophies, the dystroglycanopathies, which appear to share the primary biochemical mechanism of hypoglycosylation of alpha-dystroglycan, with decreased ability to bind its extracellular matrix ligands (laminin and agrin). Specific mutated genes and their resultant disorders include Large (congenital muscular dystrophy type 1D), fukutin (Fukuyama-type congenital muscular dystrophy), fukutin-related protein (congenital muscular dystrophy type 1C and limb girdle muscular dystrophy type 2I), and protein-O-mannosyltransferase 1 (Walker-Warburg Syndrome).

The scope of the dystroglycanopathies exceeds the skeletal muscle consequences, as multi-system effects (e.g., eye and brain) are frequent in this group of disorders. One example is Walker-Warburg syndrome, which is characterized by congenital muscular dystrophy and a wide range of other organ system symptoms. It is likely that the glycosyltransferases implicated in muscular dystrophy have targets in addition to the ubiquitously expressed alpha-dystroglycan and that these may contribute to the observed phenotypic diversity.

Calpainopathies

Calpain 3 is mutated in limb girdle muscular dystrophy type 2A. Calpain 3 is the muscle-specific member of the calcium dependent protease family. Diseases that result from deletions, translocations, and point mutations in calpain 3 have been identified in patients with limb girdle muscular dystrophy type 2A. Most of the disease-causing mutations reduce either protein expression level or protease activity.

Identification of calpain 3 localization and binding partners. Calpain 3 is believed to be localized in the cytosol, although it has never been successfully immunolocalized due to the non-specificity of available antibody reagents. Some of the endogenous substrates of calpain 3 have been identified and these include talin, ezrin, and filamin. Titin is both the substrate and the only known binding partner of calpain 3. Association with titin may be important for stabilizing the enzyme and regulating its activity. Mutations in titin that result in muscular dystrophy produce destabilization and reduced accumulation of calpain 3. Furthermore, some mutations in the calpain 3 gene that are associated with limb girdle muscular dystrophy type 2A result in loss of anchorage of calpain 3 to titin.

Morphological features of the calpainopathies differ from other muscular dystrophies. Pathogenesis in calpain 3 mutations appears to be different from other limb girdle muscular dystrophies since a compromised plasma membrane is not a feature of the disease; paradoxically, patients with calpain 3 mutations exhibit elevated serum creatine kinase. While there is some muscle degeneration in patients lacking calpain 3, calpainopathy is mainly a wasting disease with atrophy as a key component. Biopsies of limb girdle muscular dystrophy type 2A patients show accumulation of subsarcolemmal NFKB and elevated apoptosis. Muscle biopsies also show areas of degeneration and regeneration, abundant, disorganized mitochondria, and lobulated fibers.

Development of mouse models for calpainopathy. Mice lacking calpain 3 replicate the phenotypic features of limb girdle muscular dystrophy type 2A, including atrophy, small areas of degeneration/regeneration, disorganized mitochondria, and lobulated fibers, but do not exhibit apoptosis as an early feature of the pathology. In addition, calpain 3 knockout mice show abnormal sarcomere formation and organization. Mechanistically, calpain 3 appears to act upstream of the ubiquitin ligases. In turn, their role is likely to release myofibrillar and cytoskeletal proteins from the organized myofibrillar complex. Insoluble protein aggregates have been detected in calpain 3 knock out muscles suggesting that calpain deficiency is likely a disease of excessive protein accumulation rather than excessive protein degradation.

Defects in Membrane Repair

Dysferlin mutations cause muscular dystrophy and myopathy. Dysferlin is a transmembrane protein that is mutated in limb girdle muscular dystrophy type 2B and an allelic variant, Myoshi myopathy. The finding that the identical dysferlin mutation causes these two different muscle disease phenotypes supports a role for genetic background/modifier genes in the pathogenesis of the dysferlinopathies. Recent studies show that dysferlin functions in membrane repair and that defective repair represents a novel mechanism of muscle degeneration. In contrast to many of the other muscular dystrophies, where the mutated protein leads to loss of sarcolemmal structural integrity, the dysferlinopathies then are a consequence of compromised sarcolemmal repair mechanisms.

Dysferlin distribution is altered in dystrophin-glycoprotein complex-based muscular dystrophies. Dysferlin is normally associated with the sarcolemma, but is displaced to the cytoplasm in

several types of muscular dystrophy. In the converse situation of dysferlin mutations, the dystrophin-glycoprotein complex is not disrupted.

Emergence of the concept of a membrane repair complex. Evidence suggests that dysferlin may be one component of a complex or pathway that is necessary for sarcolemmal repair. It has been demonstrated that dysferlin interacts with caveolin 3. Caveolins are proteins associated with invaginations of the plasma membrane; caveolin 3 is specifically expressed in muscle. Mutations in caveolin 3 cause limb girdle muscular dystrophy type 1C and rippling muscle disease. Dysferlin also interacts with annexin 1 and 2, which are calcium and phospholipids binding proteins involved in clustering vesicles and lipid rafts. A more complete understanding of the proteins and the molecular mechanisms of muscle membrane repair may accelerate advances in muscular dystrophy research.

Oculopharyngeal Muscular Dystrophy

The PABPN1 protein is prone to formation of intranuclear aggregates. Patients with oculopharyngeal muscular dystrophy exhibit characteristic nuclear inclusions specific to skeletal muscle cells. Since PABPN1 appears to function in multiple cell types, the mechanisms behind the skeletal muscle specificity are unclear. Expansion of the CGC repeat in PABPN1 from 10 to greater than 12 copies has been associated with the disease. Recent studies have shown that normal PABPN1 is inherently aggregation-prone when expressed in an in vitro model. Cytoplasmic displacement of overexpressed normal or mutant PABPN1 reduces intranuclear aggregate formation. Muscle pathology may not only be due to nuclear aggregation of PABPN1, as microarray studies have shown that over 200 genes are overexpressed following PABPN1 overexpression, with several overexpressed proteins co-aggregating with PABPN1. Finally, studies show that PABPN1 protein is not irreversibly sequestered into aggregates; the dynamic state of the intranuclear aggregates suggests that there are potential avenues for treatment even well after disease onset.

PABPN1 protein aggregates may be mechanistic in oculopharyngeal muscular dystrophy. The appearance of nuclear aggregates of PABPN1 protein correlates with cellular toxicity in oculopharyngeal muscular dystrophy. PABPN1 aggregates are degraded by the ubiquitin-proteosome pathway; the activity of this pathway may explain the late onset of the disease and pathway inhibition has been shown to increase aggregate formation in an in vitro model. In cell-based models, inhibition of the ubiquitin-proteosome pathway with inducers of the chaperone, HSP70, reduced aggregate formation and cell death.

Development of a transgenic model of oculopharyngeal muscular dystrophy. Transgenic expression of human PABPN1 containing the normal (10 alanines) or disease-causing (17 alanines) number of repeats targeted to skeletal muscle of mice yielded skeletal muscle-specific pathology resembling that in oculopharyngeal muscular dystrophy patients. Transgenic mice with the high residue number presented with progressive muscle weakness that was accompanied by intranuclear aggregates and apoptotic muscle nuclei. Reduction of the polyalanine aggregate burden with doxycycline treatment resulted in a substantial delay and attenuation of histopathology in the mouse model.

C. RESEARCH OBJECTIVES

There is compelling evidence that the precipitating mechanisms and pathogenesis of the various types of muscular dystrophy exhibit both disease-specific and conserved properties. A comparative understanding of the convergence and divergence of pathogenic cascades that lead to muscle degeneration will help identify the best targets for therapeutic development in single muscular dystrophies, as well as potentially allow the emergence of novel therapies with

applicability to more than one disease. For all of the muscular dystrophies, how the primary disease mechanisms interact with the ongoing myogenic cell-based regeneration also should be carefully considered, as the status of endogenous regeneration has the potential to substantially affect the design, timing, and efficacy of treatments.

Disease-Specific Pathogenic Mechanisms

The initial genetic mechanisms that are responsible for the various types of muscular dystrophy are diverse, ranging from single gene mutations to the expansion or contraction of DNA repeat sequences that, in turn, may alter the expression or post-transcriptional processing of other genes. An understanding of disease-specific pathogenic mechanisms may accelerate the development the most optimal therapies.

- *Research Objective 1:* Define the role that disrupted signal transduction pathways play in the pathogenesis of Duchenne and Becker muscular dystrophies and assess their viability as therapeutic targets (Long Term; Low Risk).

Rational drug design to specific molecular targets, particularly cell signaling pathways, has proven successful in a variety of disorders such as cancer and atherosclerosis. Recent studies suggest that there is an opportunity to develop dystrophinopathy treatments that have their basis in signal transduction pathways that either support muscle cell survival or lead to muscle cell death. As in other diseases, aberrant signaling may be responsible for some of the deleterious consequences of muscular dystrophy. To this end, additional effort should be directed toward defining the downstream signaling pathways controlled by components of the dystrophin-glycoprotein complex and understanding which are affected in the dystrophin-glycoprotein complex-based muscular dystrophies. The roles of altered nNOS and NFKB signaling may be particularly important. Studies of models of muscle atrophy and regeneration also may facilitate the identification of signaling cascades that are potential therapeutic targets.

- *Research Objective 2:* Define post-transcriptional mechanisms that lead to muscle (and other tissue) phenotypes in myotonic dystrophy and evaluate the role of DNA repair/replication/recombination mechanisms in microsatellite expansion (Medium Term; Intermediate Risk).

Studies should be initiated to clarify the potential roles of RNA mis-splicing, and other post-transcriptional/translational processes in myotonic dystrophy as they relate to abnormalities of skeletal muscle, the brain (cognitive impairment, hypersomnolence, effects of personality and behavior), endocrine/other systems (frontal balding, hypogammaglobulinemia), the visual system (cataracts), cardiac muscle, and the skeleton (talipes).

Understanding the relative roles of DNA mismatch repair, recombination, and replication in microsatellite expansion is relevant to dissecting the mechanisms underlying myotonic dystrophy. Research objectives should include defining the role of DNA mismatch repair proteins in the mechanism of triplet repeat expansion, testing chemical modifiers of instability in mouse models, and creating tools that allow for the rational design of therapeutic agents to modulate genetic instability.

- *Research Objective 3:* Define the molecular pathogenetic mechanisms that lead to facioscapulohumeral muscular dystrophy (Long Term; High Risk).

Defining the molecular mechanisms by which a reduction in repeats at the D4Z4 translates into the multi-system symptoms seen in facioscapulohumeral muscular dystrophy has been difficult. Elucidation of the function of the allelic variants (A and B) at D4Z4 may help advance understanding of disease mechanisms. If perturbations of chromatin structure and/or derepression of gene expression ultimately figure into pathogenesis, there are some other diseases that could help inform researchers in this field. A potentially important avenue of research is the analysis of the chromatin structure at the D4Z4 locus, including methylation and/or binding of specific repressors or activators. Such chromatin conformational changes have been suggested as a possible disease mechanism, presumably affecting the regulation of expression of other genes. Since the issue of altered regulation of genes in the vicinity of D4Z4 remains controversial, there is a need for careful studies using microarrays or other techniques, to determine if genes near the D4Z4 repeat units on chromosome 4q, or at more distant locations on this chromosome, are upregulated or downregulated in facioscapulohumeral muscular dystrophy. The expression and function of the D4Z4 gene, DUX4, should be analyzed.

The association of 4qter with the nuclear lamina and the potential role of this association upon gene expression profiles should be explored. Genetic causes for facioscapulohumeral muscular dystrophy, other than the D4Z4 contraction (such as non-chromosome 4 linked cases), should be investigated in available patients.

- *Research Objective 4:* Establish mouse (and cellular) models for facioscapulohumeral muscular dystrophy, specific to emerging candidate genes and/or disease genomics, to understand the epigenetic mechanisms and for the development of novel intervention strategies (Long Term; Intermediate Risk).

Although the pathogenic mechanism of facioscapulohumeral muscular dystrophy is unknown, it is becoming increasingly well established that epigenetic, including nuclear localization, gene expression modulation due to the D4Z4 repeat contraction is the main culprit. As such, as this mechanism is unique among the muscular dystrophies, its elucidation is instrumental to understanding the pathogenic pathways and the design of evidence-based therapeutic (and preventive) strategies. Accordingly, generation of cellular and animal model systems is important. These models should comprise not only specific *cis*-candidate genes, but also genomic models, carrying the complete chromosome 4q subtelomere. The gene-specific models can probably reflect parts of the clinical phenotype, while at best the genomic models can produce faithful models for the full facioscapulohumeral muscular dystrophy spectrum.

- *Research Objective 5:* Define the multiple divergent effects that single mutations in nuclear envelope proteins exert on different tissues in Emery-Dreifuss muscular dystrophy and other laminopathies (Medium Term; High Risk).

Advances in the molecular biology of the laminopathies are relatively recent, but provide new opportunities for understanding the mechanisms of these multi-system diseases. Elucidation of the three-dimensional structures and structure-function relationships of nuclear envelope proteins, particularly those mutated in human diseases, will aid understanding of pathogenic mechanisms. Likewise, high-resolution imaging studies of nuclei and nuclear migration in cells with lamin A/C mutations during development and in response to mechanical stress may provide critical insights. It will be important to determine the downstream cellular responses that are caused by mutations in lamins A and C and other nuclear envelope proteins, with a possible focus on characterizing cell signal transduction pathways. This information may help appreciate the diversity of the multi-system consequences of the laminopathies. Finally, statistically meaningful, exploratory studies of alterations in the transcriptome and proteome of various cell

types and tissues from animal models and human subjects with lamin A/C mutations will help evaluate alternative mechanisms not yet uncovered by other approaches.

- *Research Objective 6:* Determine the pathogenic pathways that are downstream from PABPN1 protein nuclear aggregate formation in oculopharyngeal muscular dystrophy (Intermediate Term; Medium Risk).

While a strong association has been established between intranuclear aggregation of mutant PABPN1 protein and the pathogenesis of oculopharyngeal muscular dystrophy, there is little understanding of the cellular and molecular events that trigger myofiber death. Moreover, the basis for the muscle group specificity that is so characteristic of oculopharyngeal muscular dystrophy is completely unknown. The existence of an animal model based upon validated disease mechanisms provides an excellent opportunity to better understand the basis of this disease and to, through translational research effort, develop appropriate therapeutics. This model may also facilitate the identification of biomarkers to be used in presymptomatic detection and monitoring of clinical trials.

General Pathogenic Mechanisms

The identification of mechanisms that are conserved among multiple types of muscular dystrophy may represent the most rapid and efficient means of fostering drug development for these rare disorders.

- *Research Objective 7:* Support continued gene identification and biochemical characterization of rare and understudied forms of muscular dystrophy (Medium Term; Low Risk).

The candidate gene approach has opened the door for mechanistic studies in the muscular dystrophies. Yet only about 40% of limb girdle muscular dystrophy cases show linkage to a known disease gene. These data support the continuation of linkage analysis and positional cloning efforts.

- *Research Objective 8:* Define the roles of inflammation, oxidative stress, and disrupted calcium homeostasis as primary or secondary pathogenic mechanisms in the muscular dystrophies (Medium Term; Low Risk).

Although the role of inflammation in the pathogenesis of muscular dystrophy has been controversial, emerging data support a role for inflammation in the downstream pathogenesis of at least some of the muscular dystrophies. Further advances in this field will require the identification of the specific immune effectors that contribute to pathogenesis and the pinpointing of specific therapeutic targets. There is a clear need to understand what makes dystrophic muscle more susceptible to oxidative stress, and to appreciate how this feature contributes to the pathogenesis. Methods for mitigating the effects of oxidative stress should be explored as a therapeutic approach.

- *Research Objective 9:* Characterize defects in post-translational processing and resulting pathogenetic mechanisms in the congenital muscular dystrophies and related disorders (Short Term; Intermediate Risk).

The congenital muscular dystrophies represent genetically and clinically heterogeneous neuromuscular disorders. Disruption of normal post-translational processing of alpha-dystroglycan represents a novel pathological mechanism and understanding of the protein

defects responsible for dystrophic muscle changes may yield new insights into treatment of congenital muscular dystrophy. The ectopic expression of GalNAc transferase restores the membrane localization of dystrophin-associated proteins and protects skeletal muscle from damage in dystrophin-deficient mice, thereby supporting a putative role for membrane biochemical approaches for therapeutic intervention in Duchenne muscular dystrophy. Finally, understanding the role of hypoglycosylation in non-contractile tissue involvement (e.g., brain) may be particularly critical in dissecting the underlying disease mechanisms.

- *Research Objective 10:* Define biochemical mechanisms involved in membrane instability and membrane repair in dystrophic muscle (Long Term; Low Risk).

The pathogenesis of many of the muscular dystrophies has long been thought to involve destabilization of the sarcolemma. The development of novel therapeutic approaches could be informed by an understanding of the role of dysferlin and its associated proteins in repairing membrane breaks that arise in the dystrophin-glycoprotein complex-based muscular dystrophies as a consequence of muscle contraction.

- *Research Objective 11:* Elucidate apoptotic mechanisms occurring in the muscular dystrophies as potential targets for therapeutic intervention (Long Term; Low Risk).

While muscle fiber necrosis has long been established as a final pathway in the pathogenesis of the muscular dystrophies, there is evidence that programmed cell death, or apoptosis, may play an important role. Studies are needed to define the cascade of events responsible for apoptotic muscle fiber death in muscular dystrophy. Findings that dystrophin, sarcoglycan, lamin, and emerin mutations may alter signaling pathways to increase apoptosis provides compelling evidence that apoptosis may be a conserved pathogenic mechanism in the muscular dystrophies and thus a potentially important common target for therapeutic intervention. Recent data from laminin-alpha2-deficient mice suggest that anti-apoptosis therapy may indeed be a possible route to amelioration of disease.

Infrastructure Needs

While a separate group evaluated infrastructure needs (see Infrastructure Needs for Muscular Dystrophy), specific objectives that were viewed as important to elucidating disease mechanisms are covered here.

- *Research Objective 12:* Establish invertebrate, other vertebrate, and alternative model systems to study pathogenetic mechanisms of gene/RNA/protein defects that cause muscular dystrophies in humans (Long Term; Intermediate Risk).

- *Research Objective 13:* Establish mouse models for dystrophies where none exist, and expand development of mouse models that modify disease phenotype to identify/study disease modifying genes (Long Term; Intermediate Risk).

Development of new mouse and other (invertebrate, cell-based, etc.) model organisms that better replicate the human muscular dystrophy phenotypes may allow investigators to gain better insight into disease mechanisms and provide improved means for therapeutic development. Adequate access to appropriate models represents a potential roadblock to research. Improved high-throughput, pre-clinical cell- and animal-based models would also facilitate drug efficacy testing. The identification of compounds that are effective in the treatment of muscle disease has been hindered by the lack of high-throughput systems and

easily obtainable and standardized outcome measures in mice. Development of these models, using imaging or other easily performed assays, would facilitate the identification of new therapies.

- *Research Objective 14:* Establish repositories of tissues/cells/serum from muscular dystrophy patients to allow confirmation of pathogenic mechanism data that is derived from animal studies (Short Term; Low Risk).

Animal models provide vitally important tools to dissect the pathogenic mechanisms of the muscular dystrophies, but do not fully reflect the human disease. Hypotheses and mechanistic data originating from studies of animal models therefore require validation through cell-based studies of the human disease. Repositories represent a mechanism of ensuring that researchers engaged in mechanistic studies have adequate access to human tissues.

D. MATRIX OF RESEARCH OBJECTIVES IN MECHANISMS OF MUSCULAR DYSTROPHY

	Short Term (0-3 years)	Medium Term (4-6 years)	Long term (7-10 years)
High Risk		• Define the multiple divergent effects that single mutations in nuclear envelope proteins exert on different tissues in Emery-Dreifuss muscular dystrophy and other laminopathies	• Define the molecular pathogenetic mechanisms that lead to facioscapulohumeral muscular dystrophy
Intermediate Risk	• Characterize defects in post-translational processing and resulting pathogenetic mechanisms in the congenital muscular dystrophies and related disorders	• Define post-transcriptional mechanisms that lead to muscle (and other tissue) phenotypes in myotonic dystrophy and evaluate the role of DNA repair/replication/ recombination mechanisms in microsatellite expansion • Determine the pathogenic pathways that are downstream from PABPN1 protein nuclear aggregate formation in oculopharyngeal muscular dystrophy	• Establish invertebrate, other vertebrate, and alternative model systems to study pathogenetic mechanisms of gene/RNA/protein defects that cause muscular dystrophies in humans • Establish mouse (and cellular) models for facioscapulohumeral muscular dystrophy, specific to emerging candidate genes and/or disease genomics, to understand the epigenetic mechanisms and for the development of novel intervention strategies • Establish mouse models for dystrophies where none exist, and expand development of mouse models that modify disease phenotype to identify/study disease modifying genes
Low Risk		• Support continued gene identification and biochemical characterization of rare and understudied forms of muscular dystrophy • Define the roles of inflammation, oxidative stress, and disrupted calcium homeostasis as primary or secondary pathogenic mechanisms in the muscular dystrophies	• Define the role that disrupted signal transduction pathways play in the pathogenesis of Duchenne and Becker muscular dystrophies and assess their viability as therapeutic targets • Define biochemical mechanisms involved in membrane instability and membrane repair in dystrophic muscle • Elucidate apoptotic mechanisms occurring in the muscular dystrophies as potential targets for therapeutic intervention

DIAGNOSIS AND SCREENING IN MUSCULAR DYSTROPHY

A. INTRODUCTION

Rapid and accurate diagnosis of the muscular dystrophies reduces unnecessary testing, decreases stress of uncertainty about diagnosis, allows accurate genetic counseling, and is necessary before specific disease treatments can be considered. Accurate diagnosis of the muscular dystrophies is closely related to an understanding of the molecular mechanisms underlying the disease (see the chapter on Mechanisms of Muscular Dystrophy, for a thorough discussion of this topic). Identification of the causative gene has led to specific genetic tests for many types of muscular dystrophy. For example, despite the technical hurdle of the large size of the dystrophin gene, molecular diagnosis of Duchenne and Becker muscular dystrophies through mutation analysis is now quite accurate. For other forms of muscular dystrophy, accurate and specific diagnoses require tissue-based testing. For example, abnormal staining for merosin in skin or muscle makes a diagnosis of merosin negative congenital muscular dystrophy. These tissue-based tests can also indicate a general category of muscular dystrophy. For example, abnormal glycosylation of alpha-dystroglycan in a patient with congenital muscular dystrophy can be the result of mutation in one of at least six different genes.

For other muscular dystrophies, development of precise diagnostic procedures has been more difficult. For example, in myotonic dystrophy type 2 the large size and instability of the repeat expansion have made mutation testing difficult. For facioscapulohumeral muscular dystrophy, diagnostic testing has been challenging, in large part due to the lack of understanding of the mechanisms of the disease. As the underlying causes and molecular mechanisms of the different forms of muscular dystrophy are better understood, new approaches to molecular diagnosis will likely follow. Advances in molecular technology will facilitate test development.

Streamlining testing procedures and using the least invasive tests are priorities to reduce costs and risk to patients. Minimally invasive diagnostic approaches such as muscle imaging, or testing on easily accessible tissues (blood, skin) should continue to be explored and developed. To achieve these goals, advances in technology are often needed.

Wide availability of logical testing algorithms is desirable, to decrease unnecessary pain, expense and delay in diagnosis for patients and families. Access to genetic counseling is also a critical component of diagnostic and screening systems.

Neonatal screening is technically possible for some forms of muscular dystrophy, however, a number of social and ethical issues need to be explored to decide if widespread use is feasible or desirable.

Diagnostic testing at facilities that are engaged in research will allow development of databases that record de-identified genetic and clinical data. Mutation databases and disease-specific registries will facilitate the identification of cohorts for clinical trials. Muscle biopsy samples collected during diagnostic evaluation are an increasingly limited resource. Because of the importance of these tissues for neuromuscular research, a common database of available materials is desirable.

The ultimate goal of all diagnostic and screening procedures is improved clinical management for patients. It will be important to have accurate and widely available diagnostic procedures in place if a clear benefit for early intervention in treating these diseases is demonstrated.

B. RECENT RESEARCH ADVANCES

In the past 5-10 years, there have been significant advances in diagnostic approaches to the muscular dystrophies. The identification of new genes has facilitated the development of molecular-based diagnostic testing, and new technologies have been developed to improve the diagnosis of a number of forms of muscular dystrophy. Sensitivity and specificity have not been established for some of these tests. There is also not consensus on the appropriate diagnostic algorithm in using the available tests.

Improved Molecular Diagnosis in Duchenne/Becker Muscular Dystrophy

Genetic analysis of the dystrophin gene is complicated by its large size and complex regulation (79 exons and spread over 2.2 million base pairs of genomic DNA, including 8 promoters). The latest molecular approaches to dystrophin gene analysis are now diagnostic in approximately 95% of cases. If widely applied, use of these techniques would almost eliminate the need for diagnostic muscle biopsies in Duchenne/Becker muscular dystrophy, facilitate accurate genetic counseling, and identify appropriate patients for participation in therapeutic trials that are based upon the precise type of mutation.

Clinically available tests now include approaches to identifying small or point mutations. These use an initial screening paradigm (e.g., single-stranded conformational polymorphism and denaturing high performance liquid chromatography), followed by either limited sequencing or direct sequencing (e.g., single condition amplification/internal primer sequencing or SCAIP). Multiplex ligation dependent probe amplification (MLPA) and multiplex amplifiable probe hybridization (MAPH) have improved deletion/duplication testing.

The increasing use of these tests has also defined the limits of genomic DNA-based analysis by highlighting the prevalence of intronic mutations that result in pseudoexon insertions, which may account for up to 5% of dystrophin mutations and will continue to require mRNA-based (RT-PCR) diagnostic methods.

Dysferlin Expression Testing on Peripheral Blood Monocytes

Mutations in the dysferlin gene are associated with a variety of clinical presentations; most commonly patients present with either limb-girdle weakness (limb girdle muscular dystrophy type 2B) or distal weakness (Miyoshi myopathy). Limb girdle muscular dystrophy type 2B is estimated to account for approximately 15% of the limb girdle muscular dystrophy patients in North America. As with dystrophin, mutation detection from genomic DNA has proven difficult because of the large size of the gene. The availability of a non-invasive dysferlin expression analysis in blood monocytes has facilitated the diagnosis of the dysferlinopathies, although information about the specificity and sensitivity of these tests is quite limited.

Western Blot Approaches to Diagnosis of Limb Girdle Muscular Dystrophy

Western blot analysis of dystrophin has played a role in Duchenne/Becker muscular dystrophy testing for a number of years. More recently, an expanded role for western blotting in the

diagnosis of limb girdle muscular dystrophy and congenital muscular dystrophy has been described. This appears to be most important for calpain 3 in limb girdle muscular dystrophy type 2A and dysferlin in limb girdle muscular dystrophy type 2B. Extensive work on calpain 3 has recently been published that establishes sensitivity and specificity parameters using molecular data as the gold standard for diagnosis. This work will need independent confirmation before becoming widely accepted. Similar analyses are needed for dysferlin. To accomplish this, support is needed for western blot and molecular diagnostic evaluation of a large cohort of limb girdle muscular dystrophy patients with dysferlinopathy, as identified by immunostaining. Procedures for the miniaturization of western blot techniques have also been published in recent years. Broader application of this methodology will lead to more effective use of biopsy tissues.

Identification of the Role of Alpha-Dystroglycan in Muscular Dystrophy and Use of Screening Tests for Abnormal Glycosylation

Shortly after the discovery of dystrophin and its role in Duchenne and Becker muscular dystrophies, a membrane-associated complex of proteins was discovered and characterized. Mutations in several components of this dystrophin-glycoprotein complex were subsequently determined to cause autosomal recessive subtypes of limb girdle muscular dystrophy (in particular, the sarcoglycanopathies: limb girdle muscular dystrophy types 2C, 2D, 2E, and 2F). Central proteins of the dystrophin-glycoprotein complex, alpha- and beta-dystroglycan, are encoded by a single gene, and to date, no dystroglycan mutations have been identified in patients. The absence of a class of muscular dystrophy specifically linked to dystroglycan mutations may have its basis in the finding that targeted deletion of dystroglycan in mice is early embryonic lethal.

In a surprising twist on the standard pathophysiology in the muscular dystrophies, protein deficiency or partial deficiency of dystrophin-glycoprotein complex proteins, several genes have been discovered in the past five years that encode glycosyltransferases (or putative glycosyltransferases) that are necessary for the O-mannosylation of alpha-dystroglycan. Mutations in these genes account for Fukuyama congenital muscular dystrophy, Walker-Warburg syndrome, muscle-eye-brain disease, and at least two additional forms of congenital muscular dystrophy (types 1C and 1D), and limb girdle muscular dystrophy type 2I. The pathogenesis for each of these disorders now appears to be hypo-glycosylation of alpha-dystroglycan, leading to muscular dystrophy with or without brain and eye developmental abnormalities.

The responsible genes for the glycosylation-related muscular dystrophies are reviewed in the chapter on Mechanisms of Muscular Dystrophy. Antibodies are available that recognize alpha-dystroglycan only when it is fully glycosylated, and thereby represent useful diagnostic tools for evaluating muscle biopsies either by immunostaining or western blotting. Two of these genes have common mutations that make genetic diagnosis relatively easy. In Japanese populations there is a founder retrotransposal insertion in fukutin in > 85% of mutant alleles of Fukuyama congenital muscular dystrophy patients. More than 95% of Fukuyama congenital muscular dystrophy patients carry the retrotransposon on at least one chromosome. In limb girdle muscular dystrophy type 2I populations of European descent, there is a common point mutation in the fukutin-related protein gene that accounts for nearly 80% of mutant alleles. In addition, the fukutin-related protein gene has a single coding exon that makes sequencing easy and relatively inexpensive.

Development of Mutation Testing for Myotonic Dystrophy Type 2

The myotonic dystrophy type 2 (DM2) locus was mapped to chromosome 3 (3q21.3), and the mutation was identified as an unstable (CCTG)n expansion in intron 1 of zinc-finger protein 9 gene. Whereas normal individuals have less than 30 CCTG repeats, individuals with DM2 have 75-11,000 repeats. DM2 mutation detection has proved problematic because of the large size of repeat expansion (average 5,000 repeats) and somatic instability of the expansion. Modified Southern blot protocols have an overall sensitivity of about 80%. A three-step procedure has been proposed to include: (a) PCR-based allele sizing across DM2; the presence of two normal sized alleles excludes DM2, (b) Southern blot analysis, and (c) DM2 repeat assay. The three-step procedure is highly sensitive (98%) but is not widely available. Two recently described simplified protocols, one involving pulsed-field gel electrophoresis followed by semi-quantitative Southern blot and the second utilizing long PCR, appear to be as sensitive as the three-step procedure.

Improved Diagnostic Testing in Facioscapulohumeral Muscular Dystrophy

Facioscapulohumeral muscular dystrophy is a genetically complex disease and, despite identification of the genetic mutation approximately 13 years ago, the mechanisms and specific gene or genes involved in pathogenesis are unknown. Diagnostic testing is laborious, dependent on Southern blot technology, and complex to interpret in many cases. There is a need for more streamlined testing, which will likely be advanced by an improved understanding of the pathogenesis of facioscapulohumeral muscular dystrophy.

In the past 5 years, diagnostic testing for facioscapulohumeral muscular dystrophy has become increasingly sophisticated and complex. A deletion of a 3.3 kb repeat sequence on chromosome 4 (4q35; a deletion involving the subtelomeric D4Z4 tandem repeat array) is associated with the facioscapulohumeral muscular dystrophy phenotype. This observation provided a basis for clinical diagnostic testing. The clinical use of this diagnostic test is complicated due to cross reactivity with a highly similar repeat sequence on chromosome 10q, interchromosomal exchange, heterogeneous chromosomes, and complex rearrangements that affect the binding site of a pulsed-field gel electrophoresis DNA marker (p13E11) that is used in the diagnostic test.

A major improvement in diagnosis was the discovery that the chromosome 10q fragments could be digested away (BlnI digest). Diagnosis was still complicated by the fact that it is common for individuals to have more than 2 of the 10-type or 4-type repeat sequences because of interchromosomal exchange. The next advance in the diagnostic evaluation was the development of a “dosage test” that estimates the ratio of 10-type and 4-type sequences. This allows some degree of certainty that a short (disease-associated) fragment is actually on chromosome 4.

More recently, it has been reported that the short fragment must be in a particular environment on chromosome 4q, designated 4q35A. This observation, assuming that it is confirmed by additional groups, further refines the interpretation of diagnostic testing in facioscapulohumeral muscular dystrophy.

Because of the complexity of the algorithm and mechanics of testing, there is a need for improved documentation, training, and education for professionals (physicians, neurologists, and genetic counselors) on interpretation of genetic testing for facioscapulohumeral muscular dystrophy.

C. RESEARCH OBJECTIVES

Technology for Diagnostic Testing

DNA-based diagnostic testing is now available for some major muscular dystrophies (Duchenne, facioscapulohumeral, oculopharyngeal, and myotonic dystrophy). Genetic testing should be developed for other muscular dystrophies as well. Central diagnostic labs, which are equipped to develop and make such tests readily available, could play a key role in this effort. However, other muscular dystrophies will still require muscle biopsy for diagnosis. In some cases, alternative, minimally invasive techniques are available, but are not yet in widespread use. Large studies are also needed to confirm the validity of the currently available testing paradigms.

- *Research Objective 1:* Develop minimally invasive diagnostic techniques for muscular dystrophies where appropriate (Medium Term; Intermediate Risk).

Some patients may always require diagnosis by means other than molecular analyses. Muscle biopsy has the disadvantages of being expensive, invasive, inducing anesthesia risks, and only minimal muscle may be available for testing in advanced disease. The use of minimally invasive techniques should be further developed and expanded, since these approaches are much more acceptable to patients, less expensive to perform, and valuable as diagnostic tools or as screening tests to direct subsequent DNA-based testing. Skin biopsy has been used to demonstrate target protein abnormalities in a number of muscular dystrophies. In dystrophinopathy, the absence or abnormal dystrophin expression in erector pili smooth muscle of the skin can be demonstrated by a simple skin punch biopsy. However, since this procedure was first described, whole dystrophin gene screening has been developed, rendering the skin test obsolete. Congenital muscular dystrophy with laminin alpha2 deficiency can be diagnosed by the absence of alpha2 immunostaining in skin arrector pili smooth muscles, cutaneous nerves, and corium. Absence or deficiency of collagen VI can be demonstrated in muscle or skin fibroblast cultures to diagnose Ullrich congenital muscular dystrophy. Absence of emerin protein, demonstrated in skin by immunohistochemistry, and in blood leukocytes, by immunoblot, can be used to diagnose Emery-Dreifuss muscular dystrophy. Support should be given to expanding and validating these minimally invasive techniques.

- *Research Objective 2:* Develop definitive gene tests for muscular dystrophies for which genetic testing is not yet available (Short Term; Low Risk to Long Term; Low Risk).

Novel disease genes are frequently being discovered, but clinicians often find it difficult to obtain access to genetic testing. Both clinical and basic research would be facilitated by the targeted development of useful muscle disease diagnostic testing. Particular attention should be given to supporting central diagnostic research laboratories that are integrated into the larger muscle disease research community, and have the ability to rapidly respond to the need for the development of such tests. Several research laboratories are capable of developing such testing, but lack support to do so. Such centralized testing facilities have been developed by consortia in the United Kingdom, resulting in the centralized provision of diagnostic services, to the benefit of patients and researchers.

- *Research Objective 3:* Establish mechanisms for muscular dystrophy patients to obtain accurate genetic counseling (Short Term; Low Risk).

As genetic diagnosis is increasingly available, the need for accurate genetic counseling becomes highlighted. Genetic counseling is an ethical imperative when genetic information is being provided to a patient or family, in the clinic or as part of a research effort. The creation of better literature, brochures and training materials is needed to aid patients, families, and health care professionals. In the setting of research, demonstration that genetic counseling is available should be a mandatory element prior to approval of a project and funding to support counseling should be available. The opportunity to study the settings and timing of genetic counseling and the efficacy of this counseling.

- *Research Objective 4:* Establish the specificity and sensitivity of diagnostic tests for the muscular dystrophies (Long Term; Intermediate Risk).

New diagnostic tests for muscular dystrophies are constantly being developed. In addition to new tests using established technology, there are new technologies being introduced (e.g., micro-Western blot analysis, proteomics, gene chip technology, and laser capture microscopy). These diagnostic tests move rapidly to the clinical arena because of the clinical need. The diagnostic accuracy of the tests is often based on limited published data from a single laboratory. This is true, for example, in the use of "A" and "B" alleles in facioscapulohumeral muscular dystrophy and for monocyte dysferlin expression in limb girdle muscular dystrophy type 2B. Large studies need to be supported and published to confirm the validity of diagnostic testing in muscular dystrophy. Pathologists have a long history of working together to establish quality control and optimal techniques for new tests, and it will be important to draw on this expertise and perspective. Success in achieving this goal will likely require the use of archived samples.

Resources for the Research Community Related to Diagnosis

A theme that runs through many of the goals involving diagnosis and screening in the muscular dystrophies is the need for coordination and communication between experienced neuromuscular clinicians, neuropathologists with expertise in muscular dystrophy, and molecular diagnostic laboratories. Increased coordination could result in common algorithms for diagnostic evaluation of patients and a shared database of the diagnostic tests that are available for muscular dystrophy, including genetic, muscle-based, imaging, and tests in other tissues. The muscular dystrophy research community benefits from the ready availability of molecular diagnostic testing. However, when clinical testing is performed, the aggregate data is frequently not available to the research community. Investigators should be encouraged to submit data to existing databases to facilitate sharing of this data.

- *Research Objective 5:* Encourage submission of new mutation and polymorphism data for muscular dystrophy genes to public databases (Short Term; Low Risk).

- *Research Objective 6:* Support disease-specific registries with detailed genetic, pathological and clinical information. Foster cooperation between registries, neuromuscular research centers, and academic diagnostic centers (Long Term; Intermediate Risk).

Mutation databases have considerable utility in fostering better diagnosis (i.e., evaluation of disease-causing mutations versus simple polymorphisms), facilitating genetic counseling, and promoting research (from the informative value of individual cases, such as in-frame Becker mutations aiding development of mini-/micro-dystrophin constructs, through the value of

aggregate data in studying the influence of polymorphisms/haplotypes on disease processes). Several academic groups that offer clinical or research testing services make an effort to provide public access to their well-annotated databases containing a variety of de-identified information. Such databases exist for a variety of disorders, and include the Leiden Muscular Dystrophy pages (www.dmd.nl), the Utah Genome Center Dystrophin Mutation Tables (http://www.genome.utah.edu/DMD/mutation_tables.cgi), and the Inherited Peripheral Neuropathies Mutation Databases (http://www.molgen.ua.ac.be/CMTMutations/).
In contrast, commercially available testing organizations do not, in general, routinely present information on novel mutations, which may shed light on disease pathogenesis or approaches to therapy. Academic testing centers have, in general, demonstrated greater commitment to this goal. Databases developed and annotated by consortia of researchers should be encouraged and supported in order to provide stakeholders with accurately annotated and easily accessible information useful for diagnostic, genetic counseling, and research purposes. Such databases, in association with natural history information, will be of benefit to the muscular dystrophy community as a whole.

- *Research Objectives 7-9:* Optimize utilization of muscle biopsy materials for research by:
    - Developing IRB language to assist investigators with use of archived or prospectively collected diagnostic muscle biopsies (Short Term; Low Risk).
    - Fostering shared use of stored and prospectively collected diagnostic muscle biopsies by the research community (Short Term; Low Risk).
    - Further developing new techniques for evaluation of muscle samples (such as Western blot analysis of very small quantities, proteomics, and laser capture microscopy) and, where appropriate, moving them into the diagnostic algorithm (Medium Term; Intermediate Risk).

Muscle biopsy material is a precious resource that is often in limited supply for the research community. This will be increasingly true as non-invasive or minimally invasive diagnostic approaches are developed. There is not currently a readily accessible supply of either control muscle or muscle from patients with a genetically confirmed diagnosis. General algorithms for the appropriate diagnostic evaluation of patients with muscle disease and for handling of biopsy specimens should be agreed upon, maintained in a readily accessible format and updated regularly. Implementing this goal will require several steps.

First, neurologists and pathologists need to be educated about the appropriate diagnostic evaluation of patients and handling of muscle biopsies, including what samples to harvest and provision for long-term storage of frozen muscle for possible future diagnostic evaluation and research. General algorithms for the appropriate diagnostic evaluation of patients with muscle disease should be agreed upon, maintained in a readily accessible format and updated regularly. Such algorithms could be posted on the website suggested below, or on the Senator Paul D. Wellstone Muscular Dystrophy Collaborative Research Center websites.

Some centers already have stored muscle biopsy materials collected over many years for diagnostic purposes. It would facilitate the use of this stored muscle for research, if uniform national protocols, guidelines, or language for the use of archived material was available for submitting to various Institutional Review Boards (IRBs). While IRBs vary, having some model language posted would reduce one hurdle in using archived muscle. It may also be helpful to

have available to researchers and clinicians model IRB language to get permission to use prospectively collected muscle in research.

Shared use of these stored materials would be greatly facilitated by the development of a common set of data about the muscle samples in an integrated national database for query by investigators with specific questions. To set up such a database would require support for the data entry and support for database maintenance. In addition, there would have to be guidelines about information required prior to release of muscle.

For continued investigator access to tissues and patients, and to continue to develop improved approaches to diagnostic evaluation, it is critical that diagnostic testing remain within the academic realm. For this to happen, it is important that different labs offering pathological or genetic evaluation be integrated so that the clinician doesn't have to collect multiple samples to be sent sequentially to different laboratories.

Muscle biopsy has the potential for contributing to diagnostic evaluation and understanding of pathophysiology through emerging technology. These include Western blot analysis of very small quantities, proteomics, and laser capture microscopy. Further development of these techniques should be supported and, where appropriate, moved into the diagnostic algorithm.

Muscle Imaging as a Diagnostic Tool

It is often difficult to distinguish between an inherited dystrophic process and an acquired myopathy on clinical grounds alone. This is of utmost importance, since patients for whom the diagnosis is not clear may receive unnecessary and potentially toxic treatment or may have curative treatments delayed or withheld. While there is potential for distinguishing the muscular dystrophies that have sarcolemmal instability from those that do not, there is considerable debate as to whether modern imaging techniques will prove valuable in the diagnosis of specific types of muscular dystrophy (e.g., is MRI better than a clinical exam at distinguishing the different dystrophy types?). Even if imaging does not emerge as a widely useful diagnostic tool, it has potential for therapeutic monitoring in clinical trials.

- *Research Objective 10:* Develop and validate the role of muscle imaging in diagnostic evaluation or as an endpoint measure for clinical trials (Long Term; High Risk).

Several methods of muscle imaging (CT, MRI, MR spectroscopy, and DEXA) have been utilized in the inherited and acquired myopathies. Both CT and MRI are being utilized to identify areas of greatest pathology in order to improve the yield of muscle biopsies. This is mainly applicable to the inflammatory myopathies, where the pathology is often spotty and multifocal; muscle imaging also can identify sarcolemmal damage. Muscle imaging (MRI, CT, DEXA) can also be used to measure regional or total body muscle mass as a surrogate outcome measure in clinical trials. There have also been several studies suggesting that patterns of skeletal muscle involvement determined by MRI may be useful in distinguishing among the different muscular dystrophies. Most of these studies looked at MRI patterns in a single muscular dystrophy or compared small sample numbers in two dystrophies. It is not clear at this point how sensitive such a procedure would be, given the phenotypic variability within a given dystrophic process, and more importantly, whether imaging is more sensitive than a thorough bedside neuromuscular exam in discerning a characteristic pattern of involvement.

Priorities for future research in muscle imaging include clarifying the sensitivity and specificity of muscle imaging in muscular dystrophies from inflammatory myopathies, and in accurately and reliably distinguishing between specific muscular dystrophies.

Epidemiology Studies Based on Genetic Diagnosis

Knowledge about incidence and prevalence of genetically confirmed types of muscular dystrophy is valuable in developing reasonable algorithms and novel treatments for specific diagnoses. As specific genetic tests become available, we have the opportunity to determine the epidemiology of these diseases.

- *Research Objective 11:* Establish current and accurate incidence and prevalence data for genetically confirmed forms of muscular dystrophy (Long Term; Intermediate Risk).

Efforts are underway to determine the epidemiology of Duchenne/Becker muscular dystrophy through the MDSTARnet project funded by the CDC. The system could be expanded to include additional muscular dystrophies. A multicenter effort to characterize a large cohort of limb girdle muscular dystrophy patients will be submitted for publication in the near future. These efforts need to be expanded in other forms of muscular dystrophy.

Neonatal Testing for Muscular Dystrophy

Advances in technology have made neonatal screening approaches feasible. Neonatal screening for Duchenne muscular dystrophy, however, raises many issues. Duchenne muscular dystrophy is treatable, but not yet curable, and the treatment (corticosteroids) has only been proven efficacious in older individuals. Potential benefits to the families and patients include the shortening of the diagnostic odyssey, the opportunity for family and lifestyle planning based on the diagnosis, and the opportunity of participation in presymptomatic clinical trials aimed at early intervention strategies. Potential burdens include the psychological burden of knowledge, in a presymptomatic child, of the presence of an incurable condition, potential ramifications to insurance status, and issues of consent.

- *Research Objective 12:* Develop methods for newborn screening of the muscular dystrophies. Explore the social and ethical issues involved in offering neonatal screening for muscular dystrophy and develop techniques that would make screening practical (Long Term; Intermediate Risk).

Efforts to address some of these issues have been initiated by NICHD with the support of the Centers for Disease Control, but these activities need continued support. A consensus among stakeholders should be attempted, including determining parental attitudes toward neonatal testing. Another goal is to develop sensitive and specific methods for neonatal diagnosis using recent improvements in technology. An example is the development of genomic and proteomic methods for the screening and diagnosis of Duchenne muscular dystrophy based on blood spots. The CDC has several activities underway to research the complex issues related to newborn screening for Duchenne/Becker muscular dystrophy. CDC conducted an expert review panel to identify the research priorities and issues related to newborn screening for Duchenne/Becker muscular dystrophy. CDC currently funds a pilot newborn screening project, with emphasis on studying the challenges related to informed consent in the neonatal period. In addition, CDC is funding a pilot program for infant screening for Duchenne/Becker muscular dystrophy in pediatricians' offices. CDC funds a national survey effort that will collect information on the attitudes of parents of males with Duchenne/Becker muscular dystrophy

toward newborn screening for Duchenne/Becker muscular dystrophy. CDC is also funding a research project to study how parents of health newborn males weigh the different factors related to newborn screening for conditions without an effective early treatment such as Duchenne/Becker muscular dystrophy.

D. MATRIX OF RESEARCH OBJECTIVES IN DIAGNOSIS AND SCREENING IN MUSCULAR DYSTROPHY

	Short Term (0-3 years)	Medium Term (4-6 years)	Long term (7-10 years)
High Risk			Develop and validate the role of muscle imaging in diagnostic evaluation or as an endpoint measure for clinical trials
Intermediate Risk		• Develop minimally invasive diagnostic techniques for muscular dystrophies where appropriate • Optimize utilization of muscle biopsy materials for research by further developing new techniques for evaluation of muscle samples (such as Western blot analysis of very small quantities, proteomics, and laser capture microscopy) and, where appropriate, moving them into the diagnostic algorithm	• Establish the specificity and sensitivity of diagnostic tests for the muscular dystrophies • Support disease-specific registries with detailed genetic, structural and functional information regarding phenotypic effects in other organ systems, as well as pathological and clinical information. Foster cooperation between registries, neuromuscular research centers, and academic diagnostic centers • Establish current and accurate incidence and prevalence data for genetically confirmed forms of muscular dystrophy • Develop methods for newborn screening for the muscular dystrophies. Explore the social and ethical issues involved in offering neonatal screening for muscular dystrophy and develop techniques that would make screening practical.
Low Risk	• Develop definitive gene tests for muscular dystrophies for which genetic testing is not yet available • Establish mechanisms for muscular dystrophy patients to obtain accurate genetic counseling • Encourage submission of new mutation and polymorphism data for muscular dystrophy genes to public databases • Optimize utilization of muscle biopsy materials for research by developing IRB language to assist investigators with use of archived or prospectively collected diagnostic muscle biopsies • Optimize utilization of muscle biopsy materials for research by fostering shared use of stored and prospectively collected diagnostic muscle biopsies by the research community		

THERAPY OF MUSCULAR DYSTROPHY

A. INTRODUCTION

In the last five years, there have been some important advances in development of therapeutics for the muscular dystrophies. However, in terms of improved management of muscular dystrophy patients, the advancement has been slow, with very little of the promise from animal efficacy studies actually translating into new treatments for patients. As a result of continued questions about treatment, even the use of corticosteroids in Duchenne muscular dystrophy, which is considered standard of care, occurs in only 60% of the patients. The ability to obtain genetic diagnoses in many of these diseases has enhanced the possibility for good clinical trials, especially in diseases with phenotypic variation. The continued challenges of finding treatments and proceeding with clinical trials are multiple. Despite these challenges, there are now more potential therapies being explored at the level of preclinical studies and clinical trials than ever before. The strategy of maintaining this broad-based, parallel pursuit of the most promising therapies for the muscular dystrophies is a vitally important research objective.

Challenges faced in preclinical development for the muscular dystrophies include the difficulty of establishing and maintaining good animal models. Preclinical studies have yielded important advances in understanding some of the key muscle growth factors and their signaling pathways, as well as the regulatory mechanisms underlying muscle fibrosis. These aspects of the pathogenesis of muscular dystrophy may represent among the most directly addressable targets for novel therapy development. Other advances include identification of those muscle progenitor cells with the best potential for cell-based therapies, a rapid evolution in technologies for gene correction or gene-based therapy, identification of targets for indirect gene complementation through understanding of genes that may play complementary roles to those in the dystrophin-glycoprotein complex, and a better understanding of the gene constructs, promoters, and delivery systems needed for viral vector therapies. While animal studies have been invaluable in therapeutic development efforts, efforts have to be taken to determine if strategies that delay onset of disease in mice also slow progression in humans when administered after the onset of symptoms.

The establishment of guidelines for respiratory care for patients with Duchenne muscular dystrophy, both practice-based and evidence-based, in cooperation with the American Thoracic Society, clearly can enhance quality of life in a more uniform fashion for many of these patients. This approach sets an important standard for the multidisciplinary management of the muscular dystrophy patient.

The continued development and application of possible therapeutics to patients with a variety of muscular dystrophies will require focus on a variety of areas. With the convergence of therapeutic insights and technologies, the ability to obtain precise molecular diagnoses of disease, and the synergism provided by joint efforts of academic researchers, small and large pharmaceutical companies, and support from federal agencies and non-profit organizations, there is considerable opportunity and promise for the emergence of new, effective therapies for the muscular dystrophies. Beyond the identification of targets for the development of new therapies, there is a real need to reassess the strategies for moving from mechanistic understanding to clinically effective treatments. Investigators and funding agencies should consider a research paradigm shift, at least in part, from the hypothesis-driven approach that has been vital for key discoveries in pathogenic mechanisms to a goal-driven approach

designed to select and focus upon those therapeutic strategies with the best potential and move them forward into clinical trials.

Regularly updated information about Federally and privately supported clinical research in human volunteers is available at: http://www.clinicaltrials.gov. ClinicalTrials.gov includes information about a trial's purpose, who may participate, locations, and phone numbers for more details.

B. RECENT RESEARCH ADVANCES

There are few effective treatments available for muscular dystrophy. Current efforts to develop novel therapies include both generalized and gene-specific strategies. Generalized strategies aim at common points in the pathogenesis of muscular dystrophy, and their products may have therapeutic potential for several of the muscular dystrophies. These strategies include sarcolemmal stabilization, sarcolemmal repair, and enhancement of normal muscle regenerative mechanisms. By contrast, gene-specific strategies rely upon detailed knowledge of the molecular defect in a particular type of muscular dystrophy and thus their benefits are likely to be disease-specific; these include the replacement or repair of a defective gene or use of pharmaceuticals/small molecules to bypass the mutation site and produce a truncated, but adequately functional protein. Recently, there has been substantial effort to translate animal model efficacy data into clinical trials for muscular dystrophy.

Corticosteroid Use

Corticosteroids have become the standard of care for Duchenne muscular dystrophy, acting to delay the loss of independent ambulation, delay the onset of cardiac and respiratory failure, and reduce the occurrence of skeletal defects. Approximately 60% of Duchenne muscular dystrophy patients in the USA are taking corticosteroids. Although practice parameters have been published, several competing treatment regimens are in use in USA, Canada, and Europe, including prednisone (0.75 mg/kg/day), deflazacort (0.9 mg/kg/day), and intermittent deflazacort (10 days on/10 days off, 0.75 mg/kg/day). There is unequivocal evidence that both prednisone and deflazacort are beneficial over a 6-month period, based on double-blind controlled clinical trials; the two drugs appear to be equally effective in head-to-head studies. Retrospective analysis suggests that corticosteroids can prolong ambulation by 2 to 3 years, but the numbers of patients walking longer than the norm is not well defined. Difficulties in management of side effects remain a barrier to corticosteroid use in many patients. A phase III clinical trial to determine whether a high-dose weekly course of prednisone therapy is safer than and at least as effective as daily dose therapy in Duchenne muscular dystrophy is being conducted by an international consortium (The Cooperative International Neuromuscular Research Group (CINRG)).

Muscle Growth Factor Modulation

Myostatin inhibition. Myostatin is an endogenous negative regulator of muscle growth. The rationale for therapeutic development based upon myostatin is that muscle growth inhibitor blockade could enhance muscle regeneration. In the past five years, the myostatin signaling pathway has been elucidated, thereby providing multiple sites for drug targeting. Inhibiting myostatin has been shown to induce muscle regeneration in chronic injury models, including the mdx mouse. Since the function of myostatin is conserved across species, myostatin inhibition may have beneficial effects in human muscle disease.

A phase I/II multicenter clinical trial using a myostatin blocking antibody (MYO-029) has been started (Wyeth Pharmaceuticals). Subjects with several types of muscular dystrophy (Becker muscular dystrophy, autosomal recessive limb-girdle muscular dystrophy, and facioscapulohumeral muscular dystrophy), with differing pathogenic mechanisms, have been entered into the myostatin trial; inclusion criteria are restricted (18 years of age or older and ambulatory patients only). While a blocking antibody may have only limited applicability in chronic muscle disease, the parallel translational and clinical studies will provide an important test of the myostatin strategy and support development of alternative means of blocking the myostatin signaling cascade (e.g., with orally bioavailable small molecules).

Insulin-like growth factor 1. Following a strategy that is the converse to myostatin inhibition, the therapeutic potential of upregulating a positive regulator of muscle development and regeneration, insulin-like growth factor 1 (IGF1), has been extensively studied in the mdx mouse. Transgenic overexpression of mIGF1 in mdx muscle fibers reduces the breakdown of dystrophic muscle during the acute phase of degeneration and increases muscle mass. Subcutaneous injection of IGF1 in mdx mice reverses exercise-related fatigue and improves contractile function. Collectively, its combined activity of promoting muscle regenerative capacity and preventing necrosis makes IGF1 particularly attractive as a potential treatment for muscular dystrophy. A recent study in dystrophic mice showed that a combinatorial strategy, gene therapy with both dystrophin and IGF1, represents a clear improvement over results obtained with the dystrophin gene construct alone. A clinical trial of IGF1 linked to IGF binding protein 3 (SomatoKine), is planned for myotonic dystrophy patients (University of Rochester). Due to its regeneration-enhancing mechanism, SomatoKine may have general applicability as a treatment for the muscular dystrophies. In addition, discovery programs are ongoing to find small molecules that can upregulate muscle-specific IGF1.

Cell-Based Therapy

The goal of cell-based therapy designs is to repopulate dystrophic muscle with cells that are capable of either fusing with existing muscle fibers, or forming muscle fibers de novo, and then go on to express the defective gene. The cell-based therapy strategies that are currently under investigation differ in the source of muscle precursor cells.

Myoblast transplantation. Muscle is a regenerative organ; upon activation, a resident satellite cell population forms muscle precursor cells or myoblasts. Although ongoing regeneration is a characteristic feature of muscular dystrophy, it ultimately fails to match the progressive muscle degeneration. Genetically normal myoblasts can be isolated from normal subjects, expanded in culture, and then delivered by direct injection into dystrophic muscles. The rationale for this approach is that muscle function may be restored provided that sufficient numbers of normal myoblasts are delivered, overcome muscle fibrosis, hypo-vascularity, and/or any host immune response, and proceed to form new muscle fibers.

Phase I/II trials are ongoing using myoblast transplantation. In these studies, myoblasts are derived from healthy, genetically related donors and injected into selected target muscles. Immunosuppression is a key to long-term survival of myoblasts and FK506 seems to be of benefit. However, the therapeutic potential for myoblast transfer has yet to be demonstrated. Important hurdles to the success of myoblast transplantation are: (a) identification of cell populations with the greatest potential for myogenesis, (b) the limited treatment potential of direct muscle injection (systemic delivery potential of satellite cells is poor and myoblast dispersement from single injection sites is limited), (c) initiation of treatment early enough to

avoid the complications of fibrosis and poor perfusion of the dystrophic muscle target, and (d) developing protocols to prevent rejection of transplanted cells. Recent studies suggest that autologous transfer of myoblasts from unaffected to affected muscles may have therapeutic value in facioscapulohumeral and oculopharyngeal muscular dystrophies.

Other muscle progenitor cells. Recently, additional populations of muscle progenitor cells (i.e., the side population (SP) cells in muscle and bone marrow) have been identified using cell sorting technology. It remains a considerable debate to what degree bone marrow-derived cells can differentiate into muscle. Recent studies suggest that, with Notch transcription factor modulation, bone marrow-derived cells can be induced to develop into muscle cells. Bone marrow cells may be better than the progenitor cells that reside in skeletal muscle at overcoming the hurdle of systemic delivery. Yet, key successes with bone marrow cells have failed to be reproduced in mouse models by some groups.

Progenitor cells that are derived from muscle are an active area of investigation and may hold promise. While recent studies suggest that they have considerable potential for repopulating dystrophic muscle and their in vitro expansion can yield therapeutically relevant numbers of cells for transplantation, a concern about possible transformation during in vitro expansion and subsequent tumorgenicity remains. Clearly, embryonic-derived stem cells are known to have considerable ability to differentiate and develop into muscle. Embryo-derived cells offer the additional advantage that they are likely to avoid issues related to immune-mediated rejection.

Improvement in Understanding of Pathogenesis

While advances in mechanistic understanding of the muscular dystrophies are reviewed in the chapter on Mechanisms of Muscular Dystrophy, two specific advances that may rapidly drive therapeutic development are summarized here.

Glycosyltransferases. Sarcolemmal proteins, such as alpha-dystroglycan, link the sarcolemma to the extracellular matrix through binding of its carbohydrate moieties with the basal lamina proteins (in the case of alpha-dystroglycan, to laminin alpha2). The enzymes responsible for modifying cell surface glycoproteins and glycolipids have recently been recognized to be implicated in limb girdle and congenital muscular dystrophies. Glycosyltransferase deficiency may also play a significant role in hereditary cardiomyopathy. Fukutin-related protein (FKRP) mutations result in limb girdle muscular dystrophy type 2I and congenital muscular dystrophy type 1C. It is estimated that limb girdle muscular dystrophy type 2I is one of the most common forms of limb girdle muscular dystrophy worldwide. Therapies addressing the hypo-glycosylation of alpha-dystroglycan may have value independent of replacement of the defective gene.

Dysferlin and its role in muscle membrane repair. Mutations in the gene encoding dysferlin are common causes of limb girdle muscular dystrophy, having been described in patients throughout the world. A mouse model lacking dysferlin was generated and the skeletal muscle from this mouse displays abnormal resealing of laser-generated tears in the plasma membrane. A number of genetic defects that lead to muscular dystrophy arise from defects of plasma membrane stability, and these defects include mutations in dystrophin and the sarcoglycans. In contrast, mutations in dysferlin lead to muscular dystrophy by a defect in plasma membrane repair. Therefore, this represents a novel mechanism for muscular dystrophy. Therapy aimed at stabilizing muscle plasma membrane may be less effective for dysferlin-mediated dystrophy. Understanding how membrane repair occurs could lead to therapy for many forms of muscular dystrophy. Dysferlin is a key molecule in dissecting this process.

Advances in Genetic Diagnosis

Most dystrophin mutations that cause Duchenne muscular dystrophy are large deletions or insertions that cause codon reading frameshifts (58%) or small frameshifts or point mutations (41%), with the remainder due to duplications. About a third of new Duchenne muscular dystrophy patients have de novo mutations, obviating the need for diagnosis and screening programs. There has been substantial progress in structure-function studies of dystrophin. Not all domains of dystrophin are equally essential for generating a functional protein. Mutations leading to the absence of spectrin repeats in the rod domain, but with C-terminal and cysteine-rich domain preservation, may cause a milder Becker muscular dystrophy. The understanding of molecular structure-function from basic science studies and recent progress in genotype-phenotype correlations represent an important base for therapeutic development.

A precise molecular diagnosis (i.e., exact information on the nature of the mutation) is essential for many of the putative therapies that are moving into clinical trials. Advances in sequencing of the dystrophin gene have expanded clinical diagnostic capabilities, now including at least 30% of patients with Duchenne muscular dystrophy that might otherwise be excluded from clinical trials. Gene sequencing of the dystrophin gene has expanded clinical diagnostic capabilities and allowed identification of small mutations, including nonsense mutations sought for clinical trials. The genetic basis of the majority of the muscular dystrophy and congenital myopathies is now known. Through a combination of commercially available testing and research laboratories, genetic diagnosis is now also available for the majority of patients. Other advances in molecular diagnosis of the muscular dystrophies, and the Research Objectives for this area, are compiled in the chapter on Diagnosis and Screening in Muscular Dystrophy.

Viral Vector Gene Replacement Therapy

Several clinical trials designed to replace defective genes in Duchenne muscular dystrophy or autosomal recessive limb girdle muscular dystrophy are in planning stages (Columbus Children's Research Institute, National Institute of Neurological Disorders and Stroke, and Asklepios Biopharmaceuticals). Considerable progress has been made in gene therapy constructs, construct delivery vectors, and promoter optimization.

Micro-dystrophins for AAV somatic gene therapy. Due to its large size, full length dystrophin constructs cannot be accommodated in AAV vector. To circumvent this problem, systematic studies have identified micro- and mini-dystrophins that are functional in mdx mice. This approach began with the identification of a truncated dystrophin in a Becker patient that was still ambulatory in the 7th decade of life. Other smaller dystrophins have been described in mild Becker patients and there is evidence for a deletional "cold spot" 5' to the intron 44, with the suggestion that ascertainment bias has precluded the clinical recognition of asymptomatic in-frame (and "in-phase") deletions. These Becker muscular dystrophy mini-dystrophins (and mini-utrophins) have been extensively studied in vivo in the mdx and double null (dystrophin-deficient/utrophin knock-out) mouse. Meanwhile, germline gene transfer studies have suggested that the C-terminal dystrophin binding sites for syntrophin and dystrobrevin might be "expendable". This finding has been further exploited in the construction of micro-dystrophin cassettes that subsequently have been tested in mdx mice by both germline and somatic gene transfer, notably in the context of rAAV vectors. Dystrophin constructs appropriately sized for integration into AAV, with at least 4 spectrin-like repeats, appear to have the capacity to reverse much of the histopathology and contractile dysfunction characteristic of the mdx mouse model.

There are not, as yet, any reports of micro-dystrophin construct efficacy in the large animal model.

Optimization of AAV vectors. Recombinant AAV (rAAV) vectors now have a greatly expanded repertoire, including the serotypes AAV1-AAV9. Many of these are potentially applicable for clinical trials in muscle disease. Although AAV2 is the only serotype studied in clinical trials to date, preclinical studies have suggested that the efficiency of muscle transduction may be higher with other naturally occurring and "synthetic" serotypes. Although switching serotypes will mandate an uncertain amount of additional regulatory scrutiny, improved transfection efficiency may be a compelling rationale. Most testing of the alternative serotypes has have been limited to mice or young hamsters, but they have shown promise in these systems for systemic gene transfer following intravenous delivery. In a notable recent study, dogs previously sensitized to AAV2 had efficient hepatic transduction with AAV8. This has not yet been replicated with muscle as the target tissue; likewise, transduction efficiency and potential myositis in a large animal previously exposed to the same AAV serotype has not yet been addressed. Recent data suggest that alternative methods of vector delivery may dramatically alter the relative transduction efficiencies of the various serotypes, potentially obviating the need for a switch from AAV2.

Optimization of AAV enhancers, promoters and target genes. Muscle-specific promoters (e.g., muscle-specific creatine kinase) in experimental gene therapy may ensure greater safety, although these express poorly in heart and diaphragm when compared to cytomegalovirus (CMV) promoter. Ubiquitous over-expression of utrophin is non-toxic in mice, although the levels are lower than generally expected with CMV. There is, however, another possible theoretical advantage to muscle-specific expression of AAV-encoded proteins, the potential avoidance of immune recognition of the transgene product. Direct evidence for this is limited; more recent studies using other transgenes have shown evidence for strong humeral and cellular immune responses despite the use of a muscle-specific promoter. There is also a potential trade-off between attaining high-level expression versus tissue specificity. Micro-dystrophin coding cassettes have been optimized to fit into AAV while retaining much of the protein product functionality, but leave very little room for promoter engineering.

Gene Correction/Gene-Based Therapy

Many of the mutations in dystrophin that cause Duchenne muscular dystrophy act to cause either a deletion or a premature stop codon in messenger RNA that is located within the rod/spectrin repeat domain of dystrophin. Since the important binding domains of dystrophin are preserved, these mutations are amenable to emerging gene correction technology.

Stop codon therapy. Approximately 15% of the mutations that cause Duchenne muscular dystrophy are due to point mutations in the coding sequence that cause premature termination of transcription (stop codons), leading to a truncated, non-functional protein product. Members of the aminoglycoside class of antibiotics produce readthrough of these premature stop codons to produce a nearly intact dystrophin. One of these drugs, gentamicin, increases dystrophin expression in mdx mice by 10-20%. Small clinical trials with Duchenne muscular dystrophy patients have been conducted with gentamicin with varying results (in one study, serum creatine kinase levels decreased following a 14 day course without demonstration of dystrophin expression). A new gentamicin trial is underway in Duchenne muscular dystrophy (Columbus Children's Research Institute).

Current drug development efforts are aimed at identifying orally bioavailable agents that can promote stop codon readthrough with improved pharmacokinetics and reduced toxicity. PTC124, a compound developed via a high-throughput screen for stop codon suppression activity and structural optimization by medicinal chemistry, has been shown to induce dystrophin expression and to improve functional endpoints in mdx mice. PTC124 has completed Phase I clinical testing in healthy volunteers and is currently in Phase II clinical trials (PTC Therapeutics). An observed hierarchy of responsiveness to the suppression of different stop codon types suggests that drugs may need to be matched to each of the three stop codon sequences; accordingly, back-up compounds are being examined that may offer alternative readthrough characteristics.

Modulation of transcription and translation: exon skipping. The process of RNA splicing normally assembles each of 79 exons into a dystrophin-encoding messenger RNA in skeletal muscle; alternative combinations of these exons are selected to generate other tissue-specific dystrophin isoforms (e.g., Dp71 in brain and Dp116 in peripheral nerve). Since alternative splicing depends upon recognition of exon boundaries, it is possible to manipulate the normal gene splicing mechanisms in order to prevent the transcription of the mutation-containing exon that otherwise would interfere with generation of a functional dystrophin protein. This therapeutic development strategy has been designated as exon skipping.

Targeted skipping of mutation-containing exons could theoretically benefit 70-80% of Duchenne muscular dystrophy patients, but also may have applicability in other muscular dystrophies. By skipping exons in the rod/spectrin repeat domain of dystrophin, the resulting dystrophin product would still contain the functionally essential actin- and beta-dystroglycan-binding functional domains and potentially convert a severe Duchenne to a milder Becker muscular dystrophy phenotype. A specific antisense oligonucleotide or small nuclear RNA would be needed in order to mask each mutation-containing exon. Recent advances in exon skipping therapy development include improved chemistry (i.e., 2'-O-methyl antisense oligonucleotides (2OMeAO)) and demonstration that systemic delivery can restore dystrophin expression and partially reverse the mdx phenotype. Alternatively, AAV delivery of small nuclear U7 RNA now has been shown to increase dystrophin expression in the mdx mouse and in vitro in Duchenne cell lines. Clinical trials using the antisense oligonucleotide exon skipping strategy are expected to start soon in Australia and Europe (ProSensa, Glaxo SmithKline, University of Western Australia, Imperial College of Medicine, and Leiden University).

Taken together, these designer therapies require knowledge of the specific gene defect in each patient, increasing the importance of patient registries with a precise molecular diagnosis for registrants.

Alternative Pathways (Indirect Genetic Complementation)

Several pathways are being explored for their ability to combat elements of muscle degeneration in muscular dystrophy. Nitric oxide signaling in skeletal muscle has both vasodilation and anti-inflammatory roles; these roles are lost in both Duchenne muscular dystrophy and its animal models. Transgenic overexpression of neuronal nitric oxide synthase (nNOS) 3 (NOS3) in the mdx mouse reduces muscle pathology. The precise mechanisms underlying this success are being explored. Overexpression of NOS3 has a similar effect in ameliorating fibrosis in the hearts of mdx mice. Manipulation of the cellular localization and activity level of NOS3 has potential for muscular dystrophy therapeutic development.

Increased utrophin expression, whether mediated by transgenesis or viral delivery, can improve dystrophic pathology in mdx mice. The rationale for upregulation of the endogenous utrophin gene is that utrophin serves the same function as dystrophin, but at a very restricted site, the neuromuscular junction. The upregulation of utrophin along the entire sarcolemma, effectively replacing dystrophin, is also being attempted using small-molecule approaches.

Increased expression of an alternatively spliced form of integrin alpha7, the major laminin-binding integrin of mature muscle, improves the severely dystrophic phenotype of mdx/utrophin knock-out mice. The rationale for upregulation of alpha7 integrin using small molecules is that it, like the dystrophin-glycoprotein complex, normally provides a trans-sarcolemmal linkage to the extracellular matrix, such that increasing the density of alpha7 integrin protein in the sarcolemma may functionally compensate for the projected mechanical stabilization function of dystrophin.

Other putative targets for genetic compensation or “booster gene” therapy (and their specific targets) include a disintegrin and metalloprotease 12 (ADAM12) and acetyl-N-galactosamine (GalNAc) transferase (membrane stabilization), agrin (basement membrane formation in laminin alpha2 congenital muscular dystrophy), and calpastatin (blockade of necrosis). In addition to potentially functioning as isolated therapies, genetic compensation strategies may be useful as co-therapies to increase the chance of success of cell- or gene repair-based therapies.

Respiratory Care Guidelines

Clinical management of patients with muscular dystrophy often is variable in the nature and quality of care that is provided. Much of the care is anecdotal, occurring in the absence of clear guidance from established practice parameters. Recently, consensus guidelines were published for the respiratory care of Duchenne muscular dystrophy patients. These are practice-based guidelines, as the ability to generate evidence-based guidelines was limited due to the relatively rare nature of Duchenne muscular dystrophy. The respiratory care guidelines provide precise recommendations for the timing and extent of respiratory examinations and care, from initial diagnosis through end of life directives. The process that produced these guidelines serves as a model for developing consensus practice parameters that address the multi-system involvement seen for many of the muscular dystrophies.

C. RESEARCH OBJECTIVES

Although the underlying molecular defects have been characterized for many of the muscular dystrophies, these remain among the most difficult diseases to treat. However, there are now more potential therapies for muscular dystrophy at various stages of development than ever before. In part, this is a consequence of the improved understanding of pathogenic mechanisms (see the chapter on Mechanisms of Muscular Dystrophy) and the focused efforts toward translation of this knowledge into animal efficacy studies and then clinical trials. The Research Objectives identified here include not only a focus on the key steps needed for maturation of specific strategies, but also the recognition that efforts must be broad-based, since it is unclear as to which approaches, or combination of approaches, may ultimately prove to be the most effective in the treatment of muscular dystrophy. Combination therapies that, for example, simultaneously counter muscle necrosis/fibrosis and correct or replace the mutated gene, likely represent the best opportunity for effective treatment of muscular dystrophy.

Corticosteroids and Anti-Inflammatory Drugs

Corticosteroids represent the standard of care for Duchenne muscular dystrophy and remain the only treatment that has been established as producing a functional delay in the disease course.

- *Research Objective 1:* Optimize the use of prednisone as a treatment for Duchenne muscular dystrophy (Long Term; Low Risk).

While corticosteroids represent the only beneficial treatment for Duchenne muscular dystrophy, many remaining questions regarding the use of corticosteroids have not been answered or even addressed. An extremely important remaining question is the potential benefit of corticosteroids given early in the course of Duchenne muscular dystrophy (e.g., ages 1 or 2) to establish if muscle degeneration/loss would be prevented by early administration. The side effects of the corticosteroids remain a significant prohibitory factor for some patients and physicians. Efforts to idealize drug dosing regimens and to establish the long-term benefits and side effects are important in patient management. It has been proposed that prednisone given in regimens other than daily dosing is equally beneficial to daily doses of prednisone or deflazacort, but with fewer side effects. These alternative dosing regimens require testing in a controlled trial.

The potential long-term benefits (e.g., prolonged ambulation and improved pulmonary and/or cardiac function versus their substantial side effects, especially osteoporosis and cataracts) of corticosteroids have not yet been addressed in a prospective study. One complication for such studies is the identification of validated, short-term surrogate endpoint measures that are predictive of enhanced ambulation years after the end of a clinical trial. The effects of corticosteroid use on respiratory musculature decline and cardiomyopathy should be determined in a prospective, double-blind study. The continuing value of corticosteroid use in patients who are beyond the loss of ambulation is advocated by some, but requires systematic study.

- *Research Objective 2:* Determine the mechanism of action of the corticosteroids in muscular dystrophy in order to develop new, potentially more efficacious agents (Medium Term; High Risk).

The precise mechanism by which the corticosteroids delay progression of Duchenne muscular dystrophy is unknown. Although these agents have substantial anti-inflammatory activity, it is unclear whether this represents their primary mode of action in muscular dystrophy. Recent studies suggest that deflazacort may act by calcineurin-NFAT cell signaling pathways to promote muscle cell survival. In order to improve upon what is currently the standard of care for muscular dystrophy, it is important to determine the mechanism of action of the corticosteroids in dystrophic muscle, exploit medicinal chemistry to design new drugs to enhance therapeutic potential and minimize side effects, and move these drugs into controlled clinical trials to test against the current standard of care. Although the corticosteroids did not show efficacy for facioscapulohumeral muscular dystrophy in a small pilot trial, engineering steroid structure to exploit muscle cell survival activity may yield agents that have broad spectrum efficacy for treatment of the muscular dystrophies.

- *Research Objective 3:* Examine the efficacy of existing anti-inflammatory drugs for treatment of muscular dystrophy (Medium Term; Low Risk).

Gene expression profiling has considerably broadened our understanding of the spatial and temporal properties of inflammatory cell involvement in muscular dystrophy. Completely

blocking inflammation, however, has deleterious consequences because some inflammatory cell types are essential for muscle regeneration. Several recent studies have identified specific pro-inflammatory signaling pathways and mononuclear cell types as contributors to the pathogenesis of muscular dystrophy. This specific knowledge of pro-inflammatory pathways in muscular dystrophy should be exploited in selective preclinical and clinical testing of the efficacy of Food and Drug Administration (FDA)-approved anti-inflammatory agents.

Growth Factor Modulation

Skeletal and cardiac muscles utilize a variety of cytokines and growth factors to modulate muscle precursor cell proliferation, commitment to the myogenic lineage, myoblast fusion and differentiation into myotubes, and muscle fiber growth. The balance among positive and negative growth regulators determines muscle status between the extremes of hypertrophy and atrophy. Recent knowledge of the signaling pathways that regulate myogenesis during muscle development and regeneration provides a novel opportunity for the design of new treatments for the muscular dystrophies.

- *Research Objective 4:* Expand insulin-like growth factor 1 (IGF1) preclinical studies and the scope of ongoing clinical trials, and understand IGF1's local effects in muscle and other tissues (Short Term; Low Risk).

There is significant preclinical data that IGF1 stimulates muscle regeneration and ameliorates muscular dystrophy in mouse models. However, IGF1 has effects on multiple tissues throughout the body. Restricted expression or utilization of IGF1 for treatment of muscular dystrophy is an achievable goal. Preparations of IGF1 and IGF1 binding protein 3 are currently entering clinical trials in myotonic dystrophy. Screens are ongoing for small-molecule inducers of muscle-specific IGF1. Other modes of delivery, such as adeno-associated viral vectors (AAV), in additional disease settings, should be clinically studied. There also is a clear need to understand the long-term effects of IGF1 on muscle regenerative capacity.

- *Research Objective 5:* Identify alternative mechanisms of myostatin inhibition and establish their potential as therapeutics through preclinical testing in animal models of various types of muscular dystrophy (Medium Term; Intermediate Risk).

A neutralizing antibody to myostatin has entered clinical trials in adult muscular dystrophy, including Becker, facioscapulohumeral, and some limb girdle muscular dystrophies. Muscular dystrophies at different stages and severity may have different responses to this potential growth-inducing drug, and failure or success in one disease category may not easily translate to another. This necessitates separate clinical trials for growth regulators in the different muscular dystrophies and at different stages of disease. In addition, myostatin inhibition by a neutralizing antibody requires long-term parenteral administration. Development of orally bioavailable small molecules that might inhibit myostatin production should be explored. Multiple other drug targets in the myostatin signaling pathway, such as natural or modified inhibitory complex proteins, are known and might be developed for clinical trials.

Cell-Based Therapy

The major hurdles to myoblast transfer therapy for muscular dystrophy include the lack of dispersion of cells after direct muscle injection, immune rejection, and poor cell survival. Moreover, only a small population of transplanted cells actually contributes to muscle regeneration. Use of alternative bone marrow or embryonic stem cell populations as donor cells

will require the selective induction of the skeletal muscle lineage in cultures prior to transplantation, appropriate expression of tissue-specific endogenous genes in donor cells after transplantation, and effective integration of differentiated skeletal muscle cells into the targeted muscle. Autologous cell therapy approaches are useful in some situations (potentially in the muscular dystrophies with selective muscle group targeting, such as facioscapulohumeral and oculopharyngeal, where muscle progenitor cells could be harvested from unaffected muscles) and do not have the same requirements for immunosupression. The focus for cell-based therapeutic development needs to be upon the isolation of cell populations with optimal potential to efficiently home to and successfully engraft in muscle after systemic delivery.

- *Research Objective 6:* Achievement of adequate immunosuppression to support myoblast transplant therapy (Medium Term; Low Risk)

Myoblast transfer therapy development, based upon transfer of dystrophin-competent myoblasts from the fathers of Duchenne muscular dystrophy patients, failed in the early 1990's. Recent advances support renewed efforts to exploit natural muscle regenerative mechanisms by transferring myoblasts expressing the defective gene. For myoblast-based studies, ongoing phase I/II safety studies will continue. The emphasis for the next five years should be upon determining whether sufficient cells can be transferred and remain viable so as to improve muscle function. Targeted therapy, aiming for engraftment of particular muscle groups, will likely see success. Immunosuppressive co-therapy will utilize agents useful for other organ transplantation.

If myoblast transfer therapy is to be viable for muscular dystrophy, there are several issues besides immune rejection that must be resolved. Some of these issues are common to all cell-based therapy strategies, so progress with one specific approach may aid others. In the longer term, there is a need to develop better delivery mechanisms so that therapy is not restricted to direct injection of isolated muscle groups. Treatment of young children or newborns with muscular dystrophy has the advantage of the reduced numbers of myoblast injections necessary for relatively small muscles and the improved efficacy due to the later appearance of fibrosis, but does invoke a need for newborn screening. Conversely, treatment of very young children raises a number of ethical questions should these studies begin too soon without sufficient documentation that engrafted cells can survive long term. These issues may have the consequence of limiting cell-based clinical studies to older, more affected individuals for whom this therapy may be less effective due to excessive muscle degeneration and fibrosis.

- *Research Objective 7:* Define, through basic and preclinical translational studies, the therapeutic potential of alternative muscle progenitor cells (Medium Term; Intermediate Risk).

- *Research Objective 8:* Define, through basic and preclinical translational studies, the therapeutic potential of embryonic stem cells (Medium Term; Low Risk).

Therapeutic development with alternative muscle progenitor and embryonic stem cells likely will remain animal based for several years. This belief is based on the need to determine the full capability of these cells and their optimal sources. An advantage of using progenitor or stem cells is that the potential for blood-borne delivery, which eliminates the dispersion problem seen with myoblast transfer.

Markers should be developed to facilitate isolation of cell populations with the best myogenic and engraftment potential, and these markers and cell preparations should become widely

available to researchers to advance the field. Human counterparts of those murine cells with optimal therapeutic potential should be studied in culture models. It is reasonable to expect that human transplantation studies with alternative muscle progenitor cells may commence within approximately 5 years. Combination therapies, such as pairing cell-based therapy with strategies to maximize the myogenic potential of transplanted cells (e.g., via induction of muscle-specific transcription factors or myostatin inhibition) may be required to increase the efficacy of this approach to the level where it can have a measurable functional impact for patients.

Viral Vector Gene Therapy

The barriers to successful gene therapy include identification of optimal gene constructs (i.e., functional constructs of a size that fits into delivery vectors), promoters, and AAV serotypes, resolution of problems with protein and vector immunogenicity, and scale-up to large animal models, where immune status and vascular and muscle biology represent better approximations for the subsequent human trials. As a consequence of the progress with gene constructs and muscle-specific promoters, the focus for future studies should be on the other issues identified here.

- *Research Objective 9:* Conduct large animal model testing to determine optimal AAV serotypes for human gene therapy clinical trials (Short Term; Low Risk).

Identification and testing of alternative AAV serotypes was highly successful for dystrophin-glycoprotein complex gene delivery in small animal models (e.g., the improvement of AAV6 and AAV8 over AAV2), but findings have not yet been reproduced in a large animal model. One potential mechanism for species-specific results is the possibility that the vasculature of small rodents is intrinsically leaky. Duchenne is the only muscular dystrophy with a well-established large animal genetic model and could serve as a paradigm for other muscular dystrophies. Recent studies show that limb-wide vector delivery is both safe and highly efficient in large animals when novel vascular access approaches are used. Parallel studies using isolated cardiac perfusion in situ have demonstrated similar progress. Interestingly, the isolated perfusion approaches work with both adenovirus and AAV, suggesting that it may be valuable to revisit the issues of cloning capacity and "gutted" vectors for full-length dystrophin replacement.

The large animal approaches were first pioneered in rodent systems, reiterating the need for continued, parallel investigation in models of various sizes. The canine models for Duchenne muscular dystrophy could potentially be used to address a wide range of clinically relevant endpoints (e.g., limb, respiratory, and cardiac muscle function) following either local or systemic administration of molecular therapy. There is no published study of clinical efficacy in the canine Duchenne model with any molecular therapy. In hemophilia, demonstration of efficacy and safety in the dog model has been critical to the FDA regulatory process. Hemophilia B serves as a clear example of FDA action in the context of AAV-based therapy and should guide developmental work for muscular dystrophy. Overall, it is still reasonable to expect that an approach must work first in a rodent system before large animal studies are fully justified; however, it should also be recognized that success in the small animal is not necessarily a reliable predictor of scale-up potential.

- *Research Objective 10:* Improve the efficiency of gene therapy delivery in the muscular dystrophies, while minimizing the immune response to both gene product and delivery vehicle (Medium Term; Intermediate Risk).

The initial developmental stages for gene therapy in muscular dystrophy relied upon direct injections into selected muscles. Based upon experience from these preclinical gene therapy studies, isolated muscle injection has limited therapeutic value, while development of systemic delivery poses considerable risks. Research emphasis on an intermediate approach, isolated limb perfusion: (a) allows the use of the contralateral limb as a control, (b) offers the potential to minimize liver and germ line vector delivery, and (c) faces fewer regulatory hurdles than systemic gene transfer. Viewed as an intermediate step, preclinical translational studies and clinical trials using limb perfusion can help identify any unforeseen toxicities/complications and determine the reasonable expectations for systemic therapeutic efficacy. Since isolated limb therapies can improve quality of life by extending ambulation and/or allowing retention of leg or arm/hand usage, this strategy may be viewed by researchers and patients as more than simply an intermediate step toward systemic therapy.

Since there is variable, low-level protein expression from many dystrophin gene mutations, the recognition of dystrophin as a foreign protein may vary widely from patient to patient. Hemophilia B studies have revealed clear immunological differences between point mutational and deletional nulls. While most Duchenne muscular dystrophy patients have deletions, there is only one described deletional null animal model. Since point mutational dystrophin nulls are leaky, as demonstrated at the transcriptional level, data on immune response to "non-self" proteins in the genetic disease models must be viewed with caution. Gene therapy directed at upregulation of putative compensatory proteins (e.g., utrophin and alpha7beta1 integrin) that are normally expressed in Duchenne patients would avoid this complication.

Vector capsid antigenicity has been a problem in the rAAV clinical trials. There is a clear need for model systems for investigation of the cell-mediated immune response to vector capsid antigen in muscle, and the potential role of transient immunosuppressive strategies to circumvent this response. Furthermore, studies in mice have demonstrated differences in the immune response to a given rAAV vector based on the route of administration.

- *Research Objective 11:* Evaluate the clinical endpoints needed for, and ethical issues associated with, Phase I/II gene therapy and all clinical trials (Medium Term; Intermediate Risk).

If successful molecular therapy evolves from one of the multiple strategies under development, patients with far advanced disease may be excluded because of their accumulated loss of muscle tissue, unless muscle regeneration can be enhanced. In addition, the characteristic fibrosis in dystrophic muscle may also be rate-limiting for many potential therapies unless slowed by a co-treatment. Defining validated outcome measures is essential to tracking disease course and assessing efficacy of clinical interventions. The following measures are viewed as important for the various stages of therapeutic development: cell-based (markers), animal-based (markers, imaging, histopathology, strength, and function), human subjects (imaging, markers, clinically meaningful endpoints including strength, ambulation, spine deformity, cardiac function, pulmonary function, quality of life), non-muscle endpoint determination, and pharmacokinetic studies.

The bioethical issues associated with phase I/II clinical trials require debate. These initial trials are safety studies and lack outcome measures based on clinically meaningful endpoints to patients. In these clinical studies, the potential risks may outweigh the benefits and could preclude patients from later clinical trials when technology has advanced the field to a higher level. On the other hand, these initial clinical experiments must be carried out in order to get to more meaningful studies.

Gene Repair

Two gene repair strategies are currently under investigation: pharmaceutical-based stop codon readthrough and antisense oligonucleotide-based exon skipping. Stop codon readthrough had its basis in the initial discovery that aminoglycoside antibiotics could bypass the premature stop codons that are seen in about 15% of Duchenne muscular dystrophy patients. This technology is relatively mature and is in clinical trials with both gentamicin and an oral agent (PTC124) generated through a rational drug design strategy. Studies show substantial potential for antisense oligonucleotide-based targeted removal of one or more exons from a gene transcript to skip over disease-causing mutations and produce a shortened, but in-frame, transcript. This approach may be applicable for up to 80% of Duchenne muscular dystrophy patients, warranting additional effort to move the strategy forward through translational research and clinical trials.

- *Research Objective 12:* Evaluate the safety and efficacy of stop codon readthrough and exon skipping agents through additional translational studies and clinical trials (Long Term; Intermediate Risk).

- *Research Objective 13:* Develop novel agents to improve efficacy of current gene repair strategies or to open new strategies (Medium Term; High Risk).

Important remaining steps in advancing the stop codon readthrough strategy are to complete ongoing phase I/II safety trials and move into controlled evaluation of the efficacy of the most promising agents in Duchenne muscular dystrophy patients. Depending upon the efficiency that these agents show in clinical trials, exploration of additional novel oral stop codon therapeutics may be required. As exon skipping moves into phase I/II trials, the key research issues are to refine oligonucleotide chemistry, obtain high efficiency systemic delivery, maintain continuous antisense oligonucleotide expression (improving in vivo antisense oligonucleotide stability or otherwise circumventing the need for reinjection strategies based upon oligonucleotide and dystrophin half-life), and resolve regulatory issues that now require each new oligonucleotide sequence to be treated as a separate IND.

High-Throughput Screening and Translational Research

While there is considerable mechanistic understanding of many of the muscular dystrophies, a therapy that pre-dates much of this knowledge, corticosteroids, remains the standard of care for Duchenne muscular dystrophy and there are few, if any, effective treatments for the other muscular dystrophies. Efforts should be focused upon new strategies to translate knowledge of the pathogenic mechanisms in muscular dystrophy into testable therapeutics.

- *Research Objective 14:* Identify new strategies to implement translational research projects for muscular dystrophy (Short Term; Low Risk).

Much of the substantial mechanistic data that has been developed through hypothesis-driven studies of the dystrophin-glycoprotein complex-based muscular dystrophies has yet to translate into therapeutics. There is a considerable need for the entire research community to focus efforts on the milestone-driven drug screening and therapeutic development strategies that are used by the pharmaceutical industry. By directing preclinical therapy development efforts toward those efficacy and toxicology/biodistribution studies that are necessary for FDA investigational new drug (IND) approval, candidate therapeutics can be more rapidly culled and,

as appropriate, moved into controlled clinical trials. Since no specific putative therapy has yet emerged as a "leading candidate," and combination therapy is likely to be necessary, care should be taken to ensure that development of multiple therapeutic strategies continues to proceed in parallel.

- *Research Objective 15:* Expand high-throughput, small molecule screening efforts for promising therapeutic targets and identify novel targets for drug development (Short Term; Low Risk).

Several endogenous proteins have shown efficacy in targeting steps in the pathogenesis of muscular dystrophy. These include utrophin (a structural/functional analog of dystrophin normally with very restricted expression in muscle), integrin alpha7 (a transmembrane protein that, in parallel with dystrophin, links the sarcolemma to the extracellular matrix), myostatin (a negative regulator of muscle growth; its inhibition accelerates muscle regeneration), and IGF1 (a positive regulator of muscle growth; its activation accelerates muscle regeneration).

The transcriptional start site for the major striated muscle isoform of utrophin (A) has been identified and constructs for use in promoter analysis developed. More recently, a second isoform has been identified (B-utrophin) and its promoter have been identified and cloned. A reporter fragment of the A-utrophin promoter has been engineered into a line of transgenic mice to allow for in vivo screening. Isolated promoter fragments have been used in vitro for both high-throughput and targeted screens for low molecular weight transcriptional upregulators. The role for heregulin in transcriptional regulation of utrophin was identified in this way, and therapeutic efficacy studies in the mdx mouse have provided proof-of-concept for this general approach. One European effort has focused on a high-throughput screening approach for utrophin upregulation (www.vastox.com/research/muscular_dystrophy.html), but there has been little public discussion of an analogous approach to involve US-based partners.

A priority for research is to support targeted approaches, such as that for utrophin upregulation, or consider adding incentives for industry to participate in a high-throughput screening effort directed at endogenous proteins that can counter the inherited gene defects in the muscular dystrophies. Recognition and coordination with ongoing efforts in other countries is essential to maximize the impact of limited resources.

Cardiopulmonary Care

Because many of the genes responsible for muscular dystrophy have shared functions in skeletal and cardiac muscle, patients often develop conduction block, arrhythmia, and cardiomyopathy. Standard cardiopulmonary therapy is quite effective at reducing the symptoms associated with and reducing mortality from congestive heart failure, but has not been systematically evaluated to ensure the best quality of care.

- *Research Objectives 16-18:* Improve treatment for cardiopulmonary consequences in muscular dystrophy patients by:
    - Developing guidelines based on evidence and/or current practice standard of care (Short Term; Low Risk).
    - Assessing the value of FDA-approved agents for cardiopulmonary complication prevention (Long Term; Low Risk).

- Improving management of cardiomyopathy, conduction block, and arrhythmia (Long Term; Intermediate Risk).

Practice guidelines for the cardiopulmonary care of muscular dystrophy patients should be developed, as was previously done for pulmonary care in Duchenne muscular dystrophy. Guidelines for both cardiovascular and pulmonary care should be provided to neuromuscular disorder clinics and be made readily available to patients/families through websites for health care provider/patient-based education. The absence of information on the natural history of cardiomyopathy in susceptible populations of muscular dystrophy patients also represents an important gap in knowledge.

The mainstay of therapy for cardiomyopathy in the adult population relies on angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockade. When used in combination with beta blockade, mortality is significantly reduced. Additional agents that are effective in adult populations with cardiomyopathy are spironolactone and digoxin. Diuretics also are used as needed for symptoms of fluid overload. Cardiopulmonary care in the pediatric muscular dystrophy patient has generally lagged that in adults. A recent study suggested that early institution of ACE inhibitors reduced the onset of cardiomyopathy in Duchenne muscular dystrophy. Early combination therapy (ACE inhibitor and beta blockade) would be expected to be even more beneficial and requires assessment in controlled clinical trials.

There is a real need for improved cardiac care guidelines in all muscular dystrophy patients. Guidelines for the use of internal defibrillators have been developed in the adult cardiomyopathy population, but not for pediatric muscular dystrophy patients. Cardiomyopathy patients with left ventricular ejection fraction of less than 30% are receiving implanted cardiac defibrillators. Alternative options for pediatric muscular dystrophy patients include external defibrillators. Even in the adult population of myotonic dystrophy patients, there is a real question as to when implanted defibrillators are appropriate.

Infrastructure Needs

While the needs for infrastructural support for research in the muscular dystrophies are reviewed in the chapter on Infrastructure Needs for Muscular Dystrophy, specific objectives that were viewed as particularly important to developing new therapies are covered here.

- *Research Objective 19:* Develop the animal models, assays, and tools necessary for preclinical translational research projects that focus upon rapidly moving the accumulated mechanistic knowledge into clinical practice (Medium Term; Intermediate Risk).

- *Research Objective 20:* Improve the availability and distribution network for appropriate mouse models of muscular dystrophy (Short Term; Low RIsk).

- *Research Objective 21:* Ensure the availability and use of large animal models for the later stages of preclinical development (Long Term; Low Risk).

The availability of appropriate animal models for efficacy studies and assays for high-throughput screening represents a potential obstacle to development of new treatments for the muscular dystrophies. It is at least as important to reject drugs and therapeutic strategies at an early stage, thereby saving valuable resources, as it is to identify those strategies with the most

promise to move into controlled clinical trials. Among large animal models, the deletional null dog model may allow informative preclinical studies of a wide range of molecular therapies for dystrophinopathies. There are other canine and/or feline models for laminin and sarcoglycan gene mutations that, if further developed, would provide additional disease-specific systems for critical studies in these forms of muscular dystrophy. In addition, there are models for potentially related conditions, such as cardiomyopathy, that could be optimized for translational studies, via further genetic analysis and colony expansion.

D. MATRIX OF RESEARCH OBJECTIVES IN THERAPY OF MUSCULAR DYSTROPHY

	Short Term (0-3 years)	Medium Term (4-6 years)	Long term (7-10 years)
High Risk		• Determine the mechanism of action of the corticosteroids in muscular dystrophy in order to develop new, potentially more efficacious agents • Develop novel agents to improve efficacy of current gene repair strategies or to open new strategies	
Intermediate Risk		• Identify alternative mechanisms of myostatin inhibition and establish their potential as therapeutics through preclinical testing in animal models of various types of muscular dystrophy • Define, through basic and preclinical translational studies, the therapeutic potential of alternative muscle progenitor cells • Improve the efficiency of gene therapy delivery in the muscular dystrophies, while minimizing the immune response to both gene product and delivery vehicle • Evaluate the clinical endpoints needed for, and ethical issues associated with, Phase I/II gene therapy and all clinical trials • Develop the animal models, assays, and tools necessary for preclinical translational research projects that focus upon rapidly moving the accumulated mechanistic knowledge into clinical practice	• Improve treatment for cardiopulmonary consequences in muscular dystrophy patients by improving management of cardiomyopathy, conduction block, and arrhythmia • Evaluate the safety and efficacy of stop codon readthrough and exon skipping agents through additional translational studies and clinical trials

Therapy of Muscular Dystrophy matrix is continued on next page.

	Short Term (0-3 years)	Medium Term (4-6 years)	Long term (7-10 years)
Low Risk	• Expand insulin-like growth factor 1 (IGF1) preclinical studies and the scope of ongoing clinical trials, and understand IGF1's local effects in muscle and other tissues • Conduct large animal model testing to determine optimal AAV serotypes for human gene therapy clinical trials • Identify new strategies to implement translational research projects for muscular dystrophy • Expand high-throughput, small molecule screening efforts for promising therapeutic targets and identify novel targets for drug development • Improve treatment for cardiopulmonary consequences in muscular dystrophy patients by developing guidelines based on evidence and/or current practice standard of care • Improve the availability and distribution network for appropriate mouse models of muscular dystrophy	• Define, through basic and preclinical translational studies, the therapeutic potential of embryonic stem cells • Examine the efficacy of existing anti-inflammatory drugs for treatment of muscular dystrophy • Achievement of adequate immunosuppression to support myoblast transplant therapy	• Optimize the use of prednisone as a treatment for Duchenne muscular dystrophy • Improve treatment for cardiopulmonary consequences n muscular dystrophy patients by assessing the value of FDA-approved agents for cardiopulmonary complication prevention • Ensure the availability and use of large animal models for the later stages of preclinical development

LIVING WITH MUSCULAR DYSTROPHY

A. INTRODUCTION

The muscular dystrophies are multi-system disorders affecting many body systems besides the musculoskeletal system. Individuals with muscular dystrophy experience not only the principal effects on muscle degeneration, but also many secondary conditions, some of which are serious conditions of their own. Thus, the individual's quality of life is affected not only by muscle weakness and loss of mobility, but also by conditions including bone deformities, breathing disorders, cardiomyopathy, and cognitive decline, to name just a few. Many of these conditions negatively impact one another, complicating the ability to manage the disease. Taken together, the multi-system involvement in muscular dystrophy has significant detrimental effects on the day-to-day lives of patients, and may lead to social isolation. Clearly, the "whole body" approach to the disease needs to be considered as appropriate interventions are assessed and developed.

There is currently no treatment that can stop or reverse the progression of muscular dystrophy. Other than corticosteroids, which can slow the progression of some aspects of Duchenne muscular dystrophy, most therapies are aimed at treatment of specific symptoms and focus on rehabilitation and enhancing quality of life for patients. The development of assistive technologies and specific interventions has positively impacted quality of life for many individuals, but more research is needed to develop beneficial therapeutic approaches. In particular, the benefits and risks of exercise need to be determined. One particular challenge in developing interventions is the lack of quality of life measures for these diseases. Adequate measures of quality of life and disease burden, and clinical endpoints for disease, need to be developed.

In addition to the impact of the muscular dystrophies on the patients, these debilitating diseases have a significant effect on the quality of life of families and caregivers. Beginning with an uncertain and/or lengthy diagnostic process, and continuing as the disease progresses, family members face difficult decisions and significant stresses on a daily basis. Advances in diagnosis have somewhat lessened this burden and opened the possibility of neonatal testing to allow for family planning. The availability of genetic testing means that parents and families may seek advice from genetic counselors. It is critical to provide sufficient means to educate families and improve their quality of life, in addition to managing the disease in patients themselves.

Achieving progress in the topic area of Living with Muscular Dystrophy represents a substantial challenge to the research community, in large part due to the coordination that must occur among patients and their families, multiple types of health care practitioners, various professional organizations of physicians and other health care providers, patient advocacy organizations, and governmental agencies at federal, state, and local levels. While the challenge here is to identify the best opportunities and most pressing needs, the ultimate challenge will be to achieve the degree of coordination that is necessary to achieve these objectives.

B. RECENT RESEARCH ADVANCES

Important advances have been made to improve the quality of life for muscular dystrophy patients and their families. These include reduction in the diagnostic odyssey that has been commonly associated with muscular dystrophy, better understanding of the impact of muscular dystrophy on both patients and family members, and improved diagnosis and management of the secondary consequences of these diseases. In addition, improvements have been made in a variety of interventions, including medical and surgical management, rehabilitation and allied health strategies, assistive technologies, psychosocial management, education strategies, and vocational rehabilitation. Collectively, these advances, and their associated infrastructure, create new opportunities for improving the quality of life for patients living with muscular dystrophy.

Corticosteroids in Duchenne Muscular Dystrophy

Recent reviews and practice parameter guidelines suggest that physicians should offer corticocosteroids as a treatment for Duchenne muscular dystrophy. These studies have shown that corticocosteroids significantly increase muscle strength, performance, and pulmonary function and slow the progression of weakness in boys with Duchenne muscular dystrophy. However, corticosteroids are also associated with weight gain, cushingoid facial appearance, acne, short stature, excessive hair growth and behavioral changes. More studies are needed to determine the optimal dose, dosing schedule, and age to begin treatment, in order to improve function with the least number of side effects. Studies are also needed to determine if corticosteroids have a beneficial effect on cardiac, respiratory, gastrointestinal, and cognitive function in patients with Duchenne muscular dystrophy. Better measures of quality of life and patient reported outcomes are needed to assess the effect of these compounds. Further studies are also needed to assess functional measures, in particular to better understand the milestones of disease progression.

Application of Non-Invasive Ventilation and Cough-Assist Techniques

Breathing disorders are recognized as the leading cause of mortality in neuromuscular disease. Although the natural histories of the various types of muscular dystrophy differ with respect to time course and muscle groups involved, respiratory muscle weakness in varying degrees is common to many of these diseases. Weakness in both inspiratory and expiratory muscles leads to a progressive reduction in vital capacity, and changes in the compliance of the lungs and the chest wall contribute to the restrictive pattern of pulmonary function. Over time, hypercarbia, which initially occurs only at night, is seen during the day as well. Thus, a vicious cycle of muscle weakness, nocturnal sleep disturbance, and hypercarbia leading to worsening muscle weakness ensues. Even with appropriate treatment of nocturnal respiratory disturbances, respiratory muscle weakness can progress to the point where 24 hour/day ventilatory support is needed.

Recently, chronic mechanical ventilation with either nocturnal ventilation and/or daytime intermittent ventilation has been increasingly employed in patients with more severe muscular dystrophies. This management has been shown to reduce arterial CO_2 and increase arterial O_2, decrease symptoms of respiratory failure, reduce morbidity and mortality, and improve quality of life. Twenty-four-hour per day noninvasive respiratory support has been suggested as a methodology that can prolong survival (once the maximum benefit from using nocturnal ventilation has been reached) and improve quality of life when compared with standard tracheostomy ventilation. A number of other mechanical cough-assist approaches act to

increase cough expiratory flows and help mobilize secretions. The provision of these pulmonary technologies to patients remains variable despite increasing literature supporting the use of these interventions in persons with severe restrictive lung disease due to neuromuscular weakness. The impact of such technologies on burden of care measures and quality of life remain important issues.

Early Recognition of and Advances to Treat Sleep-Disordered Breathing

During the 1980's, symptoms of sleep disordered breathing and the health risks of its late diagnosis were recognized. Non-invasive ventilation was complicated to achieve, but seemed to be associated with fewer risks than tracheostomy for neuromuscular patients. In the 1990's, new technology made non-invasive ventilation easier to use, facilitating the study of sleep hypoventilation. Advances in the technology and wider availability of the polysomnogram, as well as cheaper screening procedures, resulted in large descriptive studies showing unequivocally that early intervention with nocturnal ventilation results in many benefits for patients. Technology has influenced treatment greatly, from the availability of properly sized pediatric interfaces to the development of laptop size ventilators that are easily portable and socially acceptable. Improved study design occurred parallel to the technology development, so that decisions can now be evidence based.

Improved Techniques for Diagnosis and Testing

While advances in diagnostic testing are reviewed in depth in the chapter on Diagnosis and Screening in Muscular Dystrophy, it is important to highlight here how these advances have contributed in positive ways to "living with muscular dystrophy." New genomics and proteomics technologies have made it feasible to provide a specific diagnosis for many patients. Accurate diagnosis, in turn, has made it possible to provide life-saving treatment for some individuals—such as female Duchenne carriers with cardiomyopathy—who otherwise may not have sought treatment. The availability of accurate diagnosis also has given some individuals access to genetic counseling, although more widely available counseling is needed. In addition, the use of genetic diagnosis has opened the door for the possibility of prenatal testing. Advances in prenatal testing technology—including the use of Chorionic villus sampling, rapid PCR diagnosis of single cells removed at the blastocyst stage, and maintained viability of blastocysts following removal of a single cell—has made prenatal testing and embryo selection feasible for a wide range of conditions. Anecdotal reports suggest that this methodology has been used by several muscular dystrophy families to avoid the birth of affected children.

Early Diagnosis and Management of Cardiomyopathy

Cardiomyopathy is evident in Duchenne and Becker muscular dystrophy, myotonic muscular dystrophy, selected subtypes of limb girdle muscular dystrophy, and Emery-Dreifuss muscular dystrophy. Newer imaging approaches such as tissue Doppler echocardiography and magnetic resonance imaging have made earlier detection of cardiomyopathy in muscular dystrophies possible. Clinicians are recognizing the importance of early treatment of cardiomyopathy in Duchenne muscular dystrophy with angiotensin converting enzyme inhibitors (e.g. Enalapril, perindopril, etc.) to delay the onset and slow the progression of left ventricular dysfunction. Other agents typically used in adult cardiomyopathy such as beta blockers, diuretics, and digitalis may also have a role in the treatment of dystrophy-related cardiomyopathy, but more investigation is needed. Conduction abnormalities (such as prolongation of the PR interval, abnormal axis, and infranodal conduction abnormalities) may also be seen in myotonic muscular dystrophy. These patients may benefit from implantation of a cardiac pacemaker.

Emery-Dreifuss muscular dystrophy patients may experience atrial paralysis, atrial fibrillation, and atrial flutter. Conduction disturbances may also involve the infranodal conduction system, resulting in slow junctional escape rhythms or complete heart block. While aggressive treatment of cardiomyopathy in muscular dystrophies may improve morbidity and mortality, the impact of such treatment on functional status and quality of life are not known.

Management of Gastrointestinal Dysfunction

Swallowing disorders (dysphagia) are often associated with muscular dystrophies, with swallowing problems reported in 30-50% individuals with myotonic dystrophy, approximately 20% of individuals with Duchenne muscular dystrophy and limb girdle muscular dystrophy, and in 6% of those with facioscapulohumeral dystrophy. Slowly progressive dysphagia is one of two cardinal symptoms of oculopharyngeal muscular dystrophy (eyelid ptosis is the other). The swallowing difficulties in individuals with muscular dystrophy are frequently due to impairment of esophageal motility, which causes problems with slow transverse of the food bolus through the esophagus. Transpharyngeal transit time may be delayed as well. Some individuals may also have problems at the diaphragm associated with hiatal hernias and with reflux. Reflux can produce burning pain, difficulty with further swallowing, and may also involve aspiration pneumonia. Improved diagnosis of these conditions has occurred with the implementation of quantitative videodynamic swallowing studies, where differences in food consistency and head positioning can be studied as well as aspiration risk. Placement of a gastrostomy tube may be indicated in the case of aspiration risk. A recent analysis of the Cochrane Neuromuscular Disease Group trials register concluded that no trials have adequately evaluated treatments for dysphagia in chronic muscle disease.

Nutritional Management of Obesity and Cachexia

Muscular dystrophies are associated with the loss of skeletal muscle, gain of excess body fat, and changes in energy metabolism and physical activity over time. A reduction in 24-hour energy expenditure, decreased fat-free mass and increased fat mass in ambulatory subjects with slowly progressive muscular dystrophies has been documented. The ability to measure body composition has improved with the application of imaging techniques such as dual-energy x-ray absorptiometry, bioelectrical impedance, and magnetic resonance imaging.

Nutrition, obesity, and obesity-related conditions are understudied in all of the muscular dystrophies. It is not clear at this stage whether a single approach toward nutrition is optimal given the different age of onset, sex, and age of muscular dystrophy patients. There appears to be a high incidence of obesity in individuals with Duchenne muscular dystrophy, as well as other muscular dystrophies. Fat tissue often increases at the time of transition to a wheelchair due to reduced daily energy expenditure related to activity. Corticosteroid therapy also may be a factor. The advantages of reduction of excess adipose tissue include a lessening of the burden on already weakened muscles and potential improvement in mobility and ease of breathing. During the later stages of Duchenne muscular dystrophy, there is a rapid decline in body weight due to a hypermetabolic state. As a result, energy and protein requirements may be substantially higher during the later stages of disease. Placement of a feeding gastrostomy tube for enteral supplementation has been shown to be critical in patients with cachexia, hypermetabolic state, dysphagia, and profound weakness which limits self-feeding.

Application of Assistive Technologies

Modern assistive technology has been a major force in improving the quality of life for disabled persons. Such technology provides people with an improved means of communication, mobility, and control over their environment and gives people with complex physical disabilities an alternative way to perform many tasks that enhance independence. Great technological innovations have been achieved over the past decade, which have enormous potential for enhancing the lives of people with muscular dystrophies. To identify the technical solution needed in each case, it is essential to look at the individual's ability and the environment in which the technology will be used. Appropriate use of assistive technology (e.g., service dogs for children with muscular dystrophy) may not only enhance the individual's abilities, but may have positive, unpredicted consequences, such as helping with socialization. Awareness and knowledge of assistive technology are key factors in supporting empowerment of people with disabilities, and contribute to the success of assistive technology solutions.

Improved Understanding of Cognitive Defects

Gains in our understanding of the neuropsychological consequences of muscular dystrophies include more detailed descriptions of the neurocognitive profile. New understanding of cognitive deficits in Duchenne muscular dystrophy patients include an understanding of the general intellectual downward shift, characterization of selective verbal deficits particularly those affecting short term verbal memory, resulting consequences on reading ability, changes in developmental trajectory such that the verbal deficits are less severe with age, and observations that the relative verbal weakness occurs across intellectual level. Data demonstrating decreased brain metabolic functioning, particularly in the cerebellum and medial temporal cortices, suggests potential areas of involvement and mechanisms underlying the defects. Studies examining the associations of cognitive functioning with mutation position have suggested that distal mutations disrupting production of alternatively spliced isoforms of dystrophin (Dp71 and Dp140) are more likely to be associated with severe cognitive deficits. Associated behaviors include increased social skill deficits and anxiety and depressive symptoms. For myotonic dystrophy, profiles vary according to age of onset, such that affected children present with mental retardation while adult onset forms of disease cause dementia. In general, executive functions are preferentially impaired. Brain changes include evidence of white matter lesions, neurofibrillary tangles and increases of tau protein, as well as decreased metabolic functions in the frontal and temporal areas of the brain. Associated behaviors include increased avoidant personality. For congenital muscular dystrophy and facioscapulohumeral muscular dystrophy, the cognitive effects are less clear; reports have suggested a range of intellectual function.

Recognition of Decreased Bone Mineral Content in Duchenne Muscular Dystrophy and its Management

Bone health is frequently compromised in boys with Duchenne muscular dystrophy; fractures of long bones are more common. Fractures have negative effects on their quality of life and may contribute to the loss of ambulation. Duchenne muscular dystrophy patients also have reduced bone mineral density, which may be exacerbated by corticosteroid treatment. Corticosteroids may also increase the risk of vertebral compression fractures. Vitamin D and calcium contribute to overall bone health, but have not been proven to reduce the risk of low-impact fractures.

Issues that require further study in Duchenne muscular dystrophy include the natural history of bone health, identification of risk factors that that predict the likelihood of a low-impact fracture,

determination of the contribution of Vitamin D and calcium to bone health, and the impact of weight bearing exercise on bone strength. The role of bisphosphonates has not been documented. Developing strategies to prevent and treat reduced bone mineral density will require multicenter, collaborative studies where standardized, clinical and laboratory data on boys with Duchenne muscular dystrophy is collected. Clinical trials will hopefully document both improved bone health and quality of life for boys with Duchenne muscular dystrophy. Elucidation of the prevalence of osteoporosis in other forms of muscular dystrophy is also of critical importance.

Documentation of the Benefits of Physical Activity and Exercise in Slowly Progressive Muscular Dystrophies

Three significant problems frequently noted by persons with slowly progressive muscular dystrophies are muscle weakness, difficulty exercising, and fatigue. These issues lead to reductions in physical activity and an increased sedentary existence in muscular dystrophy populations, putting individuals at long-term risk for coronary artery disease, obesity, osteoporosis, and mental health issues such as anxiety, depression or reduced self-esteem. Reduction in functional muscle mass in individuals with neuromuscular diseases and associated functional impairments are the result of both atrophy of disuse secondary to a sedentary life style and muscle degeneration secondary to the disease. This reduction of functional muscle mass likely results in further reductions in activity levels. Endurance exercise or aerobic training may be helpful to reverse the negative effects of the de-conditioned state. Increased physical activity in persons with muscular dystrophies, through exercise or other therapeutic approaches, is likely to contribute directly to improved community locomotion, community integration, and improved ability to participate in a greater diversity of recreational options. In addition to general and preventive health benefits, physical activity may provide a number of disease-specific benefits in the disabled due to direct effects on cardiac, pulmonary, and musculoskeletal impairments.

Multi-Disciplinary Approach to Diagnosis, Treatment, and Management

In the last few years, it has been recognized that there are a number of management interventions that can alter the natural history of Duchenne muscular dystrophy and other childhood onset muscular dystrophies, so that the majority of people affected can be expected to live into adult life. Four areas that are key to the proper multidisciplinary management of muscular dystrophies are the improvement, maintenance, and support of muscle strength and function; prevention and management of spinal deformity; the management of respiratory complications; and the prevention and treatment of cardiomyopathy. To make advances in these areas, a team approach to disease management—involving neurology, physical medicine and rehabilitation, orthopedic surgery, nutrition, gastroenterology, pulmonology, respiratory therapy, cardiology, physical and occupational therapy, psychology, and genetics—is needed. Such a team approach has had an impact on the life expectancy of Duchenne muscular dystrophy over the past three decades, has reduced secondary complications in muscular dystrophies, and has likely positively affected the quality of life in affected persons and family members.

C. RESEARCH OBJECTIVES

There is a substantial burden of disease experienced by persons with muscular dystrophy and their families beginning with delayed, uncertain, or erroneous diagnosis, and continuing as the

disease progresses. The goals for research on the topic of "living with muscular dystrophy" are to reduce or eliminate this diagnostic odyssey, to improve quality of life for patients and family members, to provide for improved clinical management of primary and secondary disease consequences, and to establish mechanisms to educate patients and family, thereby reducing their psychosocial burden and isolation from society.

Quality of Life Measures in Muscular Dystrophy

Adequate quality of life and burden of disease measures for persons with muscular dystrophies and family members are essential to understand and address disease progression and rehabilitation challenges and to facilitate clinical trials.

- *Research Objective 1:* Identify and evaluate the quality of life and burden of disease measurement tools that are currently available (Short Term; Low Risk).

- *Research Objective 2:* Develop disease-specific quality of life and burden of disease measures where gaps in existing measures are found (Medium Term; Low Risk).

Many existing quality of life and burden of disease measures cover domains that are highly relevant to persons with muscular dystrophies and their family members. A web-based catalog of measures that are specific to muscular dystrophy would be useful to clinical researchers. It would include domains covered, age range, sensitivity, reliability, validity, psychometric properties, language translations (and validity of multi-lingual versions of outcome measures), and references. Disease-specific quality of life and burden of disease measures should be developed where gaps exist.

Clinical Endpoints in Natural History Studies and Clinical Trials

Current understanding of the applicability of clinical endpoints (e.g., strength, activities, function, participation, quality of life, and burden of disease) to natural history studies and clinical trials is incomplete. Due to the progressive nature of the muscular dystrophies, it is critical to understand the predictive value of specific clinical endpoints as surrogate markers of disease progression and treatment outcomes. Such means to carefully monitor disease progression over the short time periods required for clinical trials are vitally needed for all muscular dystrophies, and may be even more important in assessing therapeutics in presymptomatic and symptomatic cases of adult dystrophies with insidious onset and slow course (e.g., facioscapulohumeral and oculopharyngeal muscular dystrophies). The coordination of data collection efforts (e.g., ensuring collection of common data elements to facilitate comparison of results from different trials) will be instrumental in meeting this goal.

- *Research Objective 3:* Determine the sensitivity of clinical endpoints to changes in disease severity (Medium Term; Low Risk).

- *Research Objective 4:* Determine the magnitude of changes in endpoints which are clinically meaningful to patients and family members (Medium Term; Low Risk).

While previous clinical trials and natural history studies in muscular dystrophy have focused on impairment measures such as strength and functional testing (including timed testing), there is a need to include a broader range of clinically meaningful measures of function, participation, quality of life, and burden of disease. Data obtained from natural history studies and clinical

trials should be used to determine the sensitivity of the various endpoints to changes in clinical severity. In addition, the magnitude of changes in endpoint measures that are clinically meaningful to patients and their families should be determined.

- *Research Objective 5:* Study the interrelationship of clinical endpoints for specific muscular dystrophies (Long Term; Low Risk).

It is not known how clinical endpoints relevant to individual muscular dystrophies relate to one another. For example: what change in strength will lead to a clinically meaningful change in gait or prolongation of ambulation? What change in functional status will significantly impact quality of life? Once varied clinical endpoint data are collected longitudinally, these measures should be correlated with one another. This will allow for the determination of shorter term, clinical surrogate markers that can be used in clinical trials. For example, walking speed or distance a subject can walk may correlate highly with time to permanent reliance on a wheelchair. An intervention that can increase walking speed or distance traveled would presumably also prolong the time to wheelchair reliance. In addition, the study of the interrelationship of clinical endpoints will not only help identify which endpoints are the most clinically meaningful to patients and family members, but also provide information regarding the predictive value of specific endpoints. Assessment of the predictive value of surrogate markers may be a valuable goal for chronic corticosteroid clinical trials in muscular dystrophy.

- *Research Objective 6:* Develop standardized data collection approaches nationally using clinically meaningful, readily obtainable parameters; develop a minimum data set for national data gathering efforts (Medium Term; Intermediate Risk).

The Centers for Disease Control and Prevention has an ongoing effort to standardize data collection within the States participating in the Duchenne muscular dystrophy surveillance system. A standardized data collection approach that can be implemented nationally needs to be developed to facilitate the evaluation of treatment efficacy and prevention of secondary conditions. As more information is gathered concerning the interrelationship between clinical endpoints, the predictive value of endpoints, and the significance of endpoints to patients and family members, it should be possible to streamline data collection efforts with a focus on clinically meaningful and readily obtainable parameters. This will facilitate the development and implementation of a minimum data set for national data gathering efforts.

Consensus Guidelines for the Clinical Management of the Muscular Dystrophies

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Despite improved management of the disease, there remains a great deal of variability in the nature and quality of care provided to persons with muscular dystrophies. There is limited evidence to demonstrate the benefit of much of the care. Evidence in the form of anecdotal or retrospective clinical studies, clinical practice parameters, and/or consensus of experts regarding best practice has been developed regarding the use of corticosteroids and respiratory care. Additional consensus from clinical experts needs to be derived regarding such issues as diagnostic approaches, orthopedic management of limb and spine deformities, exercise, nutrition management, cardiac management, psychological, behavioral and educational approaches. Such recommendations need to be disseminated to physicians, other providers, affected persons, and their families to promote effective care.

- *Research Objective 7:* Develop consensus guidelines for clinical management using Duchenne muscular dystrophy as a model (Short Term; Low Risk).

- *Research Objective 8:* Develop consensus guidelines for the clinical management of other muscular dystrophies (Medium Term; Low Risk).

The Centers for Disease Control and Prevention has a project underway to develop consensus guidelines for the clinical management of Duchenne muscular dystrophy based on evidence and expert opinion. It is recognized that there have been limitations with regard to the quantity and quality of clinical research related to muscular dystrophies. While the levels of scientific evidence may not be sufficient to justify clinical practice parameters, there may still be great value in determining the state of the science and developing consensus guidelines from experts, based on their review of the literature and clinical experience. A model process for the development of consensus guidelines could be developed initially for Duchenne muscular dystrophy given the widely available clinical literature for review. Key issues for development of consensus guidelines include identification of leadership for the process, participation of a range of professional organizations (e.g., neurology, pediatrics, and cardiology), and identification of mechanisms for dissemination of consensus guidelines. This model process could then be applied to other muscular dystrophies.

Benefits and Risks of Exercise and Physical Activity

Little scientific investigation has been conducted on the effect of resistive strength training and aerobic exercise in individuals with muscular dystrophies. Largely uncontrolled and nonrandomized studies of persons with neuromuscular disorders have focused on the adaptations to strengthening exercise training, the responses to cardiopulmonary exercise testing and adaptations to aerobic exercise training, the role of de-conditioning on the responses to exercise testing and training effect, and the role of muscle fatigue and endurance in limiting physical activity. A recent analysis of the Cochrane Neuromuscular Disease Group registry and other databases suggested that moderate intensity exercise in myotonic dystrophy and facioscapulohumeral muscular dystrophy appears not to do harm, however, there is insufficient evidence to establish that it is beneficial.

- *Research Objective 9:* Determine the benefits and risks of varied exercise approaches in muscular dystrophies and develop scientifically based recommendations concerning optimal exercise, physical activity, and recreation (Medium Term; Intermediate Risk).

More comprehensive multi-center studies that examine the effect of exercise on homogeneous groups of individuals with muscular dystrophies that have been randomized to a treatment group or a control group are needed. Investigation should match for disease, age, gender, presence of concomitant diseases, severity of weakness, and physical activity level (sedentary or active). Because the individual diseases are rare and generalizations regarding the exercise response for one disease may not be applicable to another, single-subject designs may be beneficial. These studies should include comprehensive measurements of the muscular system, sensory and motor system, cardiopulmonary system, bone and connective tissue, hormonal responses, neural and endocrine system responses to exercise using the same subject pool. Studies should also focus on a comprehensive battery of longitudinal measurements of small populations as opposed to cross sectional comparisons in order to better understand the characteristics and progression of the diseases as well as the stable elements with varying levels of physical activities. Finally, as therapeutic development proceeds, it will be important to understand the effects that the interaction of a mildly effective therapy with the resulting increased ability to exercise has upon the progression of muscular dystrophy.

Understanding and Managing the Secondary Consequences of Muscular Dystrophy

Muscular dystrophies may lead to a variety of secondary health conditions that may negatively effect function, self-reported quality of life, and increase the burden of disease. Secondary conditions seen during the progression of the various muscular dystrophies may include, pulmonary complications, orthopedic deformities, reduced bone mineral content, swallowing dysfunction, impaired GI motility, cardiac de-conditioning from sedentary activity, obesity, metabolic syndrome, cognitive impairments, depression, pain, etc. Other secondary conditions may be frequently seen in myotonic dystrophy, such as endocrinopathies, hypersomnolence/ sleep disorders, and ophthalmologic problems. Early detection and availability of therapeutic options for secondary conditions will become even more important as primary disease management strategies improve.

- *Research Objective 10:* Assess the prevalence of secondary conditions in muscular dystrophy using existing longitudinal data collection efforts (Short Term; Low Risk).

The Centers for Disease Control and Prevention currently supports the Muscular Dystrophy Surveillance Tracking and Research Network (MD STARnet), which collects longitudinal data on patients with Duchenne muscular dystrophy in five states. These data will provide information on the prevalence of secondary conditions among patients with Duchenne muscular dystrophy.

- *Research Objective 11:* Assess the natural history of secondary conditions in muscular dystrophy using existing longitudinal data collection efforts (Medium Term; Low Risk).

Research should focus on determining the prevalence of secondary conditions in relation to specific disease, age, and disease severity. Elucidating the natural history of the progression of these secondary conditions will allow for the study of prevention and management strategies. This type of data is currently being collected on patients with Duchenne muscular dystrophy in Centers for Disease Control and Prevention's MD STARnet program. CDC also funds a national survey data collection effort to collect retrospective data on early natural history of Duchenne muscular dystrophy.

- *Research Objective 12:* Assess the effectiveness of clinical management approaches to prevent and treat secondary conditions using existing multicenter collaborative networks and clinically meaningful outcomes (Medium Term; Intermediate Risk).

Some of these analyses will be available for Duchenne muscular dystrophy via CDC's MD STARnet program. Clinical management strategies for many of these secondary conditions have not been subjected to rigorous prospective research designs that will yield higher levels of scientific evidence. The organization of multicenter collaborative networks for the conduct of clinical drug trials presents an opportunity for the prospective multicenter study of the effectiveness of clinical management approaches to treat secondary conditions. Examples might include the management of reduced bone mineral content, noninvasive mechanical ventilation, surgical management of spine deformity, management of lower extremity deformities, etc.

- *Research Objective 13:* Define the neuropsychological and neurobehavioral profiles that impact on quality of life and caregiver burden and identify useful interventions (Medium Term; Intermediate Risk).

Some forms of muscular dystrophy include specific neuropsychological and neurobehavioral profiles as one component of the disease. Specific impairments include learning disorders, difficulty coping with disease, stress/depression, and problems with behavior, interpersonal relationships, and community participation. The degree to which cognitive impairments may be progressive in specific forms of muscular dystrophy has not been fully determined, although recent progress in Duchenne muscular dystrophy is fueling studies of the other muscular dystrophies. Neuropsychological and neurobehavioral consequences represent a significant need in the adult muscular dystrophies as well. Because of the severity of central nervous system symptoms in myotonic dystrophy, an important clinical objective is to define the neurologic, neuropsychological, and neurobehavioral consequences of the two types of myotonic dystrophy. Finally, there is a need to develop strategies to help unaffected children cope with the stress of a parent who has muscular dystrophy and to help unaffected siblings cope with affected siblings. Research also needs to assess coping strategies and level of functioning in caregivers and identify means to ease their burdens emotionally, financially, and socially. Secondly, we need to assess interventions that may be helpful for ameliorating the learning difficulties and examine interventions known to be effective in similarly presenting disorders such as dyslexia.

Patient and Family Education and Social Participation in the Community and Physician Training

A high priority for patients and families with muscular dystrophy is access to current information related to both clinical management and potential therapeutic options for specific conditions. As individuals with muscular dystrophy enter school and subsequently move into occupations, school systems and vocational training need to be adequately staffed and equipped. Greater opportunities for education, vocation, recreation, and community integration should reduce the social isolation frequently seen in persons with more severe muscular dystrophies. Surveys of the adult muscular dystrophies show that decreasing social isolation ranks high among priorities for improving quality of life. We need to assess ways to decrease social isolation and increase acceptance into the community for individuals and families affected. Existing educational and training programs with potential applicability need to be utilized and adapted for individuals with muscular dystrophy. The breadth and depth of physician training in both management of and working with patients with muscular dystrophies has an important influence upon patient quality of life.

- *Research Objective 14:* Establish annual educational conferences for patients and families focused on specific muscular dystrophies (Short Term; Low Risk).

Educational conferences will have the greatest value when they are focused on specific muscular dystrophies. These conferences should bring together persons affected with muscular dystrophies, family members, different private advocacy groups, government organizations, clinicians, and basic science researchers with a common purpose in mind. Thus, these conferences will not only serve an educational purpose, but will have the additional benefit of promoting further cooperation and fostering collaboration among these diverse stakeholders. A successful approach for such a national conference has been developed for spinal muscular atrophy. In addition, national family conferences are currently held that focus on Duchenne and facioscapulohumeral muscular dystrophy. Future approaches to conference planning can build on recent successes at the national and regional level. Interactions among patient support groups should be facilitated to allow sharing of strategies and successes from patient conferences, so that successful conferences could be organized in the near future for Duchenne

and Becker muscular dystrophy, facioscapulohumeral muscular dystrophy, myotonic dystrophy, and limb girdle muscular dystrophy.

- *Research Objective 15:* Identify strategies to improve patient integration into educational systems (Short Term; Low Risk).

Information regarding rare diseases should be made available to school systems to assist in the provision of appropriate educational services in the least restrictive environment. Appropriate educational information and information concerning rights and responsibilities should be provided to parents so they are able to navigate the system to help their child obtain services. Without the proper assistance, tools and guidance, many children will not obtain appropriate education services or succeed. A systemic program to assess the effectiveness of the 'No Child Left Behind' and the 'Individuals with Disabilities Education Act' is a research need. Research on the effectiveness of Individualized Education Plan service delivery should integrate the resources and expertise of local public schools and the rehabilitation system. Efforts for educational integration into college and graduate programs also need to be undertaken for adult individuals with muscular dystrophy.

- *Research Objective 16:* Identify strategies to improve vocational outcomes and reduce social isolation (Medium Term; Intermediate Risk).

Vocational Rehabilitation services facilitate the transition from school or training programs to work and community living for youth and adults with significant disabilities. Successful employment outcomes and higher earnings are related to a positive relationship between the consumer and the vocational rehabilitation counselor.

- *Research Objective 17:* Develop strategies to improve physician effectiveness in communicating with and managing the care of patients with muscular dystrophy (Short Term; Low Risk).

There is a clear need for increased neuromuscular disease emphasis in neurology training programs. A new subspeciality fellowship in neuromuscular disorders has the potential to improve the care of patients with muscular dystrophy. Beyond this, efforts should be made to address the general education of neurologists and primary care physicians in best practices for diagnosis and management of the muscular dystrophies and to advance their knowledge of Federal and advocacy group resources that can reduce the burden of disease in this patient group. The medical professional organizations and specific disease organizations represent potential sources for development of educational strategies.

D. MATRIX OF RESEARCH OBJECTIVES IN LIVING WITH MUSCULAR DYSTROPHY

	Short Term (0-3 years)	Medium Term (4-6 years)	Long term (7-10 years)
High Risk			
Intermediate Risk		• Develop standardized data collection approaches nationally using clinically meaningful, readily obtainable parameters; develop a minimum data set for national data gathering efforts • Determine the benefits and risks of varied exercise approaches in muscular dystrophies and develop scientifically based recommendations concerning optimal exercise, physical activity, and recreation • Assess the effectiveness of clinical management approaches to prevent and treat secondary conditions using existing multi-center collaborative networks and clinically meaningful outcomes • Define the neuropsychological and neurobehavioral profiles that impact on quality of life and caregiver burden and identify useful interventions • Identify strategies to improve vocational outcomes and reduce social isolation	
Low Risk	• Identify and evaluate the quality of life and burden of disease measurement tools that are currently available • Develop consensus guidelines for clinical management using Duchenne muscular dystrophy as a model • Assess the prevalence of secondary conditions in muscular dystrophy using existing longitudinal data collection efforts • Establish annual educational conferences for patients and families focused on specific muscular dystrophies • Identify strategies to improve patient integration into educational systems • Develop strategies to improve physician effectiveness in communicating with and managing the care of patients with muscular dystrophy	• Develop disease-specific quality of life and burden of disease measures where gaps in existing measures are found • Determine the sensitivity of clinical endpoints to changes in disease severity • Determine the magnitude of changes in endpoints which are clinically meaningful to patients and family members • Develop consensus guidelines for the clinical management of other muscular dystrophies • Assess the natural history of secondary conditions in muscular dystrophy using existing longitudinal data collection efforts	• Study the interrelationship of clinical endpoints for specific muscular dystrophies

RESEARCH INFRASTRUCTURE NEEDS FOR MUSCULAR DYSTROPHY

A. INTRODUCTION

Research infrastructure transcends the other topics addressed by the MDCC Scientific Working Group, in that it denotes those resources that can facilitate the accomplishment of many of the other Research Objectives identified throughout this report. The Research Infrastructure Needs for Muscular Dystrophy subgroup independently examined the resources that would be necessary to accomplish the Research Objectives identified by each of the four other subgroups.

Historically, there are a number of examples where shared infrastructure and research resources facilitated a major advance in the muscular dystrophy research field. For instance, the efforts of dozens of research laboratories were carefully coordinated for the identification of the Duchenne muscular dystrophy gene, dystrophin. In this case, the research community freely exchanged clones, antibodies, samples, and testing methods in order to accelerate linkage analysis, positional cloning, and functional characterization of dystrophin. However, there are also examples where infrastructure for muscular dystrophy research has been underserved, both in terms of emphasis and funding. This is particularly true for clinical trials where the existence of clinical trial networks and research cores might have greatly facilitated the undertaking of clinical trials over the past 20 years.

Structured clinical trial networks facilitate more rapid initiation of trials by having validated endpoint measures, adequate infrastructure, sufficient patient availability, and disease-specific clinical trial expertise already in place. It would seem imperative to have operational clinical trial networks to test potential therapeutics as they emerge, particularly for Duchenne muscular dystrophy where we know a fair amount about the mechanism of disease. From 1985-1990—before the molecular basis of the disease process was determined—seven clinical trials in Duchenne muscular dystrophy, involving 396 Duchenne muscular dystrophy patients, were published, with many from the Muscular Dystrophy Association-funded Clinical Investigation of Duchenne Dystrophy (CIDD) network. The identification of the dystrophin gene and protein in 1987 resulted in an enormous advance in our knowledge of the disease mechanisms (hundreds of publications over a few years); however, these advances in knowledge did not correspond to an increase in the number of clinical trials in Duchenne muscular dystrophy. From 1991-2005, only eight trials in Duchenne muscular dystrophy, involving 122 patients, were reported, highlighting the need for infrastructure to run and manage new trials. Recently, several academic and pharmaceutical industry translational studies and clinical trials have started, exploiting the accumulated mechanistic knowledge in muscular dystrophy to explore multiple therapeutic strategies.

To address the great needs in infrastructure for muscular dystrophy basic and translational research and clinical trials, there has been a recent focus on shared research resources, including the creation of Scientific Research Resource Cores within the Senator Paul D. Wellstone Collaborative Research Centers, as well as the funding of clinical trial networks, mouse drug screening cores, high-throughput drug screening facilities, and centralized antibody production and distribution facilities.

While efforts in the USA have led to important advances in understanding the mechanisms of muscular dystrophy, recent European efforts have focused on research infrastructure, with

variable success in formation of tissue and cell banks, organization of focused meetings by the European Alliance of Muscular Dystrophy Associations, and other efforts. It is essential that international coordination occur to maximize the utilization of scare resources and ensure that unnecessary overlap is avoided.

B. CURRENTLY AVAILABLE RESEARCH RESOURCES

Resources for Mechanistic Studies

Infrastructure resources that help support basic research on the causes and pathophysiology of the muscular dystrophies were considered to encompass three distinct areas: patient sample repositories, animal models, and antibody repositories.

Patient sample repositories: DNA, cell, and tissue repositories are important, since multiple disease genes remain unknown for several muscular dystrophy phenotypes (including the mutated genes in a substantial percentage of limb girdle muscular dystrophy patients) and linkage/cloning studies require critical mass of familial genetic information that can be best facilitated by DNA repositories and the disease mechanisms that are identified in animal models require validation in human tissues that can be aided by human cell/tissue repositories.

The Italian Telethon network, the EuroBioBank, and the National Institute of Neuroscience (Kodiara, Japan) maintain tissue repositories that include the muscular dystrophies. Patient samples also are available at several large referral sites in the USA (e.g., the Universities of Rochester and Iowa and Children's National Medical Center). Biological specimen banking is also planned as part of CDC's MD STARnet program. These tissues provide an important resource for validating mechanistic data obtained in animal studies. An extensive library of biopsy specimens from neuromuscular disease patients also exists at the Institute for Neuromuscular Research (http://www.inmr.com.au/), at the University of Sydney.

Several public access DNA mutation databases contain data relevant for mechanistic studies in the muscular dystrophies, including but not restricted to: (a) Leiden University Medical Center (http://www.dmd.nl/), (b) the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/hgmd0.html), and (c) the Utah Genome Depot (http://www.genome.utah.edu/). Also, gene expression profiling databases (e.g., http://pepr.cnmcresearch.org/home.do) can provide researchers with broad-based information on pathogenic mechanisms in muscular dystrophy. Data from numerous animal and human muscular dystrophy studies are also publicly available for mining in the National Institutes of Health Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/).

Mouse and dog models: The absence or adequate access to appropriate animal models of muscular dystrophy is a potential obstacle to progress in understanding the mechanisms and pathogenesis of these diseases. Jackson Laboratory represents a substantial resource for germ-free, inbred mouse strains, including models for several neuromuscular diseases. While only those strains that are requested on a somewhat regular basis are maintained as breeding colonies with rapid availability, other strains are warehoused as frozen embryos, but access to these is not possible on a short-term basis. While many of the less frequently used strains can be obtained from research labs, supplies from these sources are inconsistent and can prove problematic in terms of acceptance by the requesting institution as germ free (i.e., often requiring re-derivation).

There is no animal model that perfectly mirrors human muscular dystrophy. The mouse models serve an important role in early therapeutic development, but dog models better reflect both disease severity and the immunological problems that are associated with several therapeutic development strategies. While dystrophic dog colonies are important for pre-clinical translational projects in therapeutic development, only limited numbers of animals are available from existing sources. To help remedy problems in investigator access to dog models, the Senator Paul D. Wellstone Collaborative Research Centers have a joint initiative to expand breeding colonies in the USA. A modern dystrophic dog facility at the National Institutes of Neuroscience (Kodaira, Japan) has a model that has not been fully explored in the USA and may represent an important, underused resource. Available dog models include the Golden Retriever (CXMD; a naturally occurring mutation that has been bred), German Shorthair Pointer (a dystrophin knockout), Welsh Corgis (a naturally occurring mutation that has been bred), and beagle (CXMDJ; artificially inseminated with frozen-thawed spermatozoa derived from an affected golden retriever). Careful comparisons of these models have not yet been done.

Muscle functional testing core facilities, for both mouse and dog, are essential to mechanistic and therapeutic development studies in the muscular dystrophies. Facilities at the University of Pennsylvania and Children's National Medical Center have developed expertise for these studies. Broader utilization of these core facilities may standardize the data that are obtained in mechanistic and therapeutic development studies.

Antibody repositories: Specific antibodies for proteins that exhibit primary or secondary involvement in the muscular dystrophies are essential for tissue- or cell-based mechanistic studies, patient diagnosis, and in helping evaluation of the efficacy of novel therapies. Most of the important antibodies are available on an ad hoc basis from the original research laboratories. As is the case for the existing animal models, access to antibodies from individual research labs can be highly variable. A specific source of many muscle disease-related antibodies is the North East Wales Institute (http://www.rjah.nhs.uk/cind/morrisge/mabs.htm), which houses about 300 mouse monoclonal antibodies, about half of which directly relate to study of the muscular dystrophies, including exon-specific dystrophin antibodies and antibodies for Emery-Dreifuss muscular dystrophy and myotonic dystrophy. The Developmental Studies Hybridoma Bank (http://www.uiowa.edu/~dshbwww/), at the University of Iowa, also is a significant, inexpensive, and rapid source of antibodies to proteins relevant to neuromuscular disease. The National Institutes of Health also supports a mouse monoclonal production facility for nervous system-related antibodies that will undertake development of antibodies for many proteins pertinent to muscular dystrophy (NeuroMab Hybridoma Facility, University of California at Davis; http://www.neuromab.org/).

Resources for Diagnosis and Screening and Therapeutic Development

Prior to 1987, the classification of the muscular dystrophies was solely based on clinical characteristics, including the pattern of inheritance, pattern of weakness, evidence of multisystemic involvement, and histological features. After identification of the dystrophin mutations that are responsible for Duchenne and Becker muscular dystrophies, the standards of diagnosis and classification began to shift to requiring refined genetic testing. Subsequent identification of genetic mutations for myotonic dystrophy, facioscapulohumeral dystrophy, oculopharyngeal muscular dystrophy, Emery-Dreifuss muscular dystrophy, and multiple forms of congenital and limb-girdle muscular dystrophy have further refined the diagnostic capabilities for these diseases. With this knowledge, the view of the muscular dystrophies began to shift toward molecular- and biochemical-based perspectives.

Identification of the responsible genetic changes has been critical both in defining the pathophysiological bases of the different forms of muscular dystrophy, and in permitting accurate diagnoses in clinical practice. Many conditions that were initially clinically defined to be distinct disorders have subsequently been shown to arise from common genetic causes, thus demonstrating the value of genetic testing. Only by use of genetic testing can the epigenetic and environmental contributions to disease phenotypes be resolved, which, in turn, might lead to the identification of novel treatment modalities.

Interpretable evaluation of therapeutic approaches depends upon the availability of sufficient numbers of accurately defined subjects. Clearly, determination of the genetic abnormality in subjects, or characterization of the clinical phenotype at the molecular level by determining abnormalities of gene product, are of immediate value in development of meaningful clinical trials. The increasing availability of accurate genetic testing for most forms of muscular dystrophy thus has set the stage for more powerful and informative clinic trials. The emergence of disease registries, such as the National Registry of Myotonic Dystrophy and Facioscapulohumeral Muscular Dystrophy Patients and Family Members (University of Rochester; http://www.urmc.rochester.edu/nihregistry/), and epidemiological studies, such as the Muscular Dystrophy Surveillance Tracking and Research Network (MD STARnet; http://www.cdc.gov/ncbddd/duchenne/mdstarnet.htm), provide an important infrastructure for supporting refinement of diagnostic and screening mechanisms and providing adequate numbers of molecularly characterized patients for clinical trials.

Many methods are used for clinical and research diagnostic testing and molecular characterization. Clinical tests are available to assess the gene product at the protein level, with immunohistochemistry or immunofluorescence being the standard of care for all muscle biopsy laboratories, and Western analysis or specialized immunostaining of some proteins available as clinical tests at specialized laboratories. Research laboratories also offer specialized testing of gene products at the protein or RNA level for further characterization of individual subjects. Genetic testing available for clinical use includes linkage analyses or mutation analyses by several different methods (e.g., PCR-based assays, entire gene sequencing, or Southern analyses) depending on the features of the gene and the type of mutation being investigated. Important advances in the tools necessary for precise and reproducible diagnosis of the muscular dystrophies are described in the chapter on Diagnosis and Screening in Muscular Dystrophy.

GeneTests (http://www.genetests.org/) is a publicly funded medical genetics information resource website that provides current, authoritative information on genetic testing and its use in diagnosis, management, and genetic counseling. The diagnostic tests that are currently available for muscular dystrophy are included in this database. There has been a proliferation of laboratories that offer genetic tests for certain forms of muscular dystrophy, most notably Duchenne (available from at least 26 labs) and myotonic dystrophy type 1 (DM1, available from at least 25 labs). Prenatal, presymptomatic, and diagnostic testing for facioscapulohumeral muscular dystrophy is available for chromosome 4-linked families through a number of academic and commercial laboratories. Genetic testing for the GCG trinucleotide repeat expansion in the PABPN1 gene that are responsible for oculopharyngeal muscular dystrophy is available from both commercial and academic labs (at least 8 labs). Other forms of muscular dystrophy are still principally diagnosed by immunostaining of muscle biopsies, with little availability of genetic testing (e.g., most forms of limb girdle and congenital muscular dystrophy). An open issue in this area relates to questions of the comparability of test results between diagnostic laboratories, and, by extension, the diagnostic frequency of the muscular dystrophies. An important aspect of testing that is hard to quantify is the degree of counseling

or interpretation that is provided to help clinicians and patients understand the full meaning of their test results.

The Cooperative International Neuromuscular Research Group (CINRG) is an international clinical trial network for studies in Duchenne/Becker muscular dystrophy. CINRG's major efforts have been been screening and testing FDA-approved drugs for efficacy in Duchenne muscular dystrophy. The Muscle Study Group (University of Rochester), a consortium of investigators interested in facilitating clinical trials in the neuromuscular disorders, also serves to coordinate clinical trials in neuromuscular disease, providing access to a considerable expertise in such studies and a data coordination center and statistical unit.

The strengths of the existing infrastructure for diagnosis and treatment of muscular dystrophy thus include:

- Diagnostic methods are now available for most forms of muscular dystrophy
- Diagnostic testing has become increasingly affordable and accessible so that a majority of muscular dystrophy patients can be accurately identified.
- Reliable testing of carriers is increasingly available for many forms of muscular dystrophy, allowing accurate genetic counseling
- Pre-natal or pre-implantation testing is increasingly available, allowing refined approaches to family planning
- Muscular dystrophy research laboratories have remained connected in many instances to the diagnostic laboratories, increasing the accuracy of information for clinicians in interpretation of results
- Patient registries and clinical networks are starting to emerge that can facilitate the conduct of clinical trials in muscular dystrophy.

Resources for Reducing the Clinical Burden of Disease

Rehabilitation research. The resources to pursue rehabilitation research and to investigate the spectrum of medical problems that are associated with the different types of muscular dystrophy are very limited. These limitations cloud the vision of the scope and type of resources necessary to accelerate research in these important areas. Rehabilitation resources can provide key preventive strategies for muscular dystrophy-associated medical problems. The National Institute on Disability and Rehabilitation Research (NIDRR) provides leadership and support for a comprehensive program of research related to the rehabilitation of individuals with disabilities, including those with muscular dystrophy. Existing infrastructure resources for rehabilitation research include the NIDRR-funded Rehabilitation Research Training Center in Neuromuscular Diseases at University of California at Davis. NIDRR also supports studies of pain, assessment of quality of life, and the effects of a community-based exercise program in neuromuscular diseases.

Quality of life. The opportunity to develop resources to study quality of life and clinical burden of disease in the muscular dystrophies is tremendous. Patients and family members are eager to pursue studies and develop the needed capabilities. To date, however, few investigations have occurred to establish the optimal instruments to measure quality of life in the muscular dystrophies, and with the growing interest in patient-reported outcomes research and in pre-emptive health care, the time is at hand to accelerate and enhance research in this area.

The National Institutes of Health sponsored a two-day workshop on the "Burden of Muscle Disease" in January 2005 to review the present status of research and the available databases and test instruments to assess quality of life and burden of disease in patients with the different types of muscular dystrophy. Additional efforts to improve the quality of life of these patients include a NIDRR-funded study, to evaluate instruments to assess quality of life in patients with muscular dystrophies. In addition, collaborative studies between CINRG, the University of California at Davis research group, and the University of Utah Dystrophin Project include instruments to assess quality of life in patients with Duchenne muscular dystrophy.
An additional study has tested instruments to measure quality of life in caregivers and family members affected by Duchenne muscular dystrophy. Finally, the Muscular Dystrophy Association has funded validation studies of the "Individualized Neuromuscular Quality of Life (INQoL) measure"—an instrument for adults with muscular dystrophy—that was developed by researchers in the United Kingdom.

Cognitive changes in muscular dystrophy. Cognitive involvement and other central nervous system dysfunction have been demonstrated in several of the muscular dystrophies. Cognitive, behavioral, and other alterations of central nervous system function occur in particularly in Duchenne muscular dystrophy and myotonic muscular dystrophy (DM1 and DM2), but our understanding of the pathophysiologic mechanisms, the impact of their deficits on learning, employment, coping with disease, and interpersonal relationships is severely limited. There are few well-established instruments to measure cognitive alterations in the muscular dystrophies and a paucity of researchers investigating these problems. For example, only a modest number of investigations of hypersomnia have occurred in myotonic dystrophy (DM1) and yet patients rate this symptom as one of the most disabling, especially as it relates to employment and meeting responsibilities in caring for children. The National Institutes of Health has funded studies of cognitive/genetic aspects and of spirituality of children with Duchenne muscular dystrophy. Opportunities abound to develop infrastructure and research studies in this area.

Resource Centers for Muscular Dystrophy

The National Institutes of Health has established six Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Centers, in order to accelerate basic and clinical research on muscular dystrophy. These Centers are located at the Children's National Medical Center, University of Iowa, University of Pennsylvania, University of Pittsburgh, University of Rochester, and the University of Washington. The goals of the Centers are to promote side-by-side basic, translational, and clinical research, to provide resources that can be used by the national muscle biology and neuromuscular research communities, and to provide training and advice about muscle diseases for researchers and physicians who provide initial diagnosis and treatment, including rehabilitation, care for cognitive and behavioral concerns, and therapy for other system complications. Taken together, the Centers constitute a cohesive program, operating under the guidelines for National Institutes of Health cooperative agreements in order to maximize collaborative utilization of the unique resources in infrastructure, expertise, and clinical recruitment that are available in the Centers.

Resources for Education and Training

The National Institutes of Health and other funding agencies are aware of the dearth of clinical investigators and the need to increase the number and scope of individuals pursuing translational research as well as basic and clinical research in muscular dystrophy. Despite having a spectrum of training opportunities for medical and graduate students, fellows, and

more established researchers the number of new investigators is lagging behind the need and opportunities.

The challenge has been to recruit and train new investigators to pursue research related to the muscular dystrophies and to educate patients and health care providers about opportunities for research and about the current standards of care for each of the different muscular dystrophies. Several initiatives in education and training have been issued by Federal agencies and patient advocacy groups. For training and research career development, the National Institutes of Health have developed Senator Paul D. Wellstone Muscular Dystrophy Collaborative Research Center fellowships (NOT-AR-05-001), Mentored Clinical Investigator Career Development Awards in Muscle Disease Research (K08 and K23; PA-05-051), Ruth L. Kirschstein National Research Service Awards for Postdoctoral Fellowships in Muscle Disease Research (F32; PA-05-052), and Academic Research Enhancement awards (R15; PA-03-053). The NIH also has established Loan Repayment Programs in the areas of Clinical Research, Pediatric Research, Health Disparities Research, and Clinical Research for Disadvantaged Background Individuals.

The Department of Education has funded the National Institute on Disability and Rehabilitation (NIDRR) Research Fellowship awards to support research on rehabilitation of individuals with disabilities. NIDRR also supports the National Center for Dissemination of Disability Research, which has sponsored the program, “Rehabilitation Science for Basic Scientists and Engineers”, to increase the number of Ph.D. engineers and basic scientists trained to perform research to solve problems of individuals with disabilities.

The Centers for Disease Control and Prevention also has initiated programs to fund educational and outreach activities in muscular dystrophy. In cooperation with CDC, Parent Project Muscular Dystrophy will conduct outreach activities targeted toward individuals who know people with muscular dystrophy (such as teachers and fellow students of children with Duchenne muscular dystrophy) and the general public. The Genetic Alliance will coordinate a CDC-funded resource center on Single Gene Disorders, and will conduct provider education activities, and disseminate resources to families, providers and the general public on Duchenne muscular dystrophy.

In addition to its regular conference grant program (R13), the NIH has initiated a targeted program to support small, focused conferences coordinated through the Senator Paul D. Wellstone Muscular Dystrophy Collaborative Research Centers (Support for Muscular Dystrophy Workshops and Research Conferences; NOT-AR-05-008). The Office of Rare Diseases also provides important support for conferences, including many related to the muscular dystrophies.

Several patient advocacy groups provide important information for patients with muscular dystrophy and their families. These include the Facioscapulohumeral Society (http://www.fshsociety.org/), the International Myotonic Dystrophy Organization (http://www.myotonicdystrophy.org/), the Muscular Dystrophy Association (http://www.mdausa.org/), the Muscular Dystrophy Family Foundation (http://www.mdff.org/), and the Parent Project Muscular Dystrophy (http://www.parentprojectmd.org). Many of these offer a variety of educational and support materials, including websites, brochures, fact sheets, and newsletters. In addition, many of these organizations fund research conferences, patient support groups, and educational projects, as well as grants to individual muscular dystrophy students, fellows, and established investigators.

Resources for Muscular Dystrophy in Europe

International cooperation and coordination is essential to achieving the goal of timely detection, diagnosis, treatment, and prevention of the muscular dystrophies. An understanding of the research strategies used by and the resources available in the European community will help ensure optimal utilization of resources.

Current funding streams for muscular dystrophy research in Europe. The European national funding bodies, like those in the USA, focus upon funding high-quality science, with only limited funding earmarked for neuromuscular diseases. The neuromuscular disease patient advocacy groups have major national differences in how much funds are generated and on the spending patterns for research and care. Scandinavian patient advocacy groups, for example, invest almost exclusively in social interventions. By contrast, the Association Française contre les Myopathies (AFM; France; www.afm-france.org) and Telethon (Italy; http://www.telethon.it/english/index.asp) are major advocacy groups with significant research budgets. Both organizations utilize peer-review systems to evaluate and fund responsive research projects and research infrastructure projects, including coordinated interdisciplinary research facilities (e.g., Genethon; http://www.genethon.fr/php/index_us.php), Neuromuscular Research Center in Paris, and Telethon Institute of Genetics and Medicine (TIGEM; http://www.tigem.it/Introduction.htm). AFM supports research in labs outside of France, while Telethon supports predominantly Italian scientists within Italy, with some support for Italian scientists working abroad who plan to return to Italy. Other charities (e.g., Muscular Dystrophy Campaign in the United Kingdom) have mixed expenditures on care and research and a very small budget.

European Union research funding is limited to expenditures on specific requests for applications. The European Union has funded specific research projects in muscular dystrophy (e.g., congenital muscular dystrophy, Bethlem muscular dystrophy, and Emery-Dreifuss muscular dystrophy) but, until recently (see below, *Addressing fragmentation in European translational research*), no strategic funding plan has existed for neuromuscular disease research.

The European Neuromuscular Centre (ENMC). The ENMC was established in 1989 through the support of the neuromuscular patient advocacy groups in Austria, Denmark, France, Germany, Italy, The Netherlands, Switzerland, the United Kingdom, and Spain. The aim of the ENMC is to foster research collaborations in neuromuscular disease. Its major activity has been support of research workshops: 137 workshops have been supported to date, involving over 1,100 participants from 30 countries. The workshops are investigator-initiated, small and tightly focused (typically < 20-25 participants). Lay reports are published immediately on the ENMC website and full meeting reports subsequently published in *Neuromuscular Disorders*. Small grants may also be awarded by ENMC.

The initial focus of the ENMC workshops was on collaborations leading to establishment of diagnostic criteria, disease gene identification, and genotype-phenotype studies. In the last few years, the ENMC has also invested in a clinical trials unit. The aims of the clinical trials unit are to promote the publication of Cochrane Reviews in Neuromuscular Diseases and facilitate the development of clinical trial applications where these are considered necessary. Focus of funding is upon initial stages of trial development, rather than being a trial management group per se. ENMC initial clinical trial priorities in muscular dystrophy were to investigate the use of steroids and the prophylactic treatment for cardiomyopathy in Duchenne muscular dystrophy.

National neuromuscular networks and service structures. Efforts have been made in various European countries to develop networks of clinicians and researchers interested in neuromuscular diseases. As with the national advocacy groups these efforts have taken different directions and have different emphases in different countries. In Germany, the opportunity to obtain Federal funding for networks addressing rare diseases allowed the development of MD-NET, which provides a national service and research structure and encompasses diagnostics, clinical services, research laboratories and a clinical trials unit. In Scandinavia, there is a well-developed network that focuses on generation of standards of assessment and reference documents for management of various neuromuscular diseases. In the United Kingdom, the Muscular Dystrophy Campaign has invested in a pediatric audit group for Duchenne muscular dystrophy (the North Star project), which is working on standardization of assessment and audit of practice standards. In Italy and France, the Telethon (with Unione Italiana Lotta alla Distrofia Muscolare, the patient organization for muscular dystrophy) and AFM are making similar efforts, and there are networks also in Belgium, the Netherlands, and Spain.

Provision of diagnostic services for the muscular dystrophies by Federal agencies is variable across Europe. In the United Kingdom, the Department of Health funds a national service that includes limb girdle muscular dystrophy and congenital muscular dystrophy. MD-NET in Germany provides a comprehensive network of diagnostic services.

Addressing fragmentation in European translational research. The European Union has focused upon infrastructure for translational research in neuromuscular disease in a recent request for applications. Specifically, networks of excellence were solicited to "aim at sharing expertise between basic and clinical academics and industrial partners in order to develop technological and methodological tools with a view to accelerate the elaboration of new therapies for rare neuromuscular diseases." These networks of excellence are designed to address fragmentation in specific research areas by integrating partners to work together on an indefinite basis, by providing funding for activities that address integration, but not the research per se. There is substantial emphasis upon infrastructure, including the development of tools, including animal models, databases, biobanks, well defined patient cohorts, and methods for efficacy assessment.

One application for support by the network of excellence focuses upon muscular dystrophy (TREAT-NMD) and exemplifies the current European Union strategy. This application proposes the establishment of a European Neuromuscular Institute committed to deliver innovative treatments for rare neuromuscular diseases from laboratory development to clinical practice. Bringing together the top researchers, clinicians, and industry researchers in Europe, this application addresses the fragmentation currently hindering the progress of promising therapies by establishing a common road map for their assessment, from cellular and animal models to clinical outcome measures. Cutting edge treatments currently under development for the muscular dystrophies will be specifically targeted in this process, while future developments will also benefit from the durable infrastructure of the Institute, which will include patient registries and biobanks. A further role of the Institute will be in the education of clinicians and researchers, thereby ensuring a long term leading position for European research in the muscular dystrophy field.

Specifically, the European Neuromuscular Institute proposal provides for: (a) acquisition of preclinical evidence for efficacy of candidate therapeutics (coordinate and develop assessment methodologies and screening techniques in different animal models and cell systems), (b) support for production and toxicology (large scale support for production of clinical grade material for clinical trials including toxicology), (c) developing and evaluating methods for

systemic delivery in humans (including commercial collaborations), (d) defining the patient population (establish, coordinate, and harmonize patient registries, databases, and biobanks in order to create a unified resource for the definition of patient cohorts and biological material), (e) harmonizing the patient population (focus on standards of care, including all aspects of multidisciplinary management with the aim to harmonize approaches to management so that defined patient groups are managed similarly, and gold standards established for testing against in future clinical trials), (f) defining outcome measures (elaborating clinical assessment tools), (g) running trials (supporting protocol design, regulatory and statistical support, liaison with orphan drugs and European Union clinical trials authorities), (h) applying the tools (coordinate program integration and bring innovative therapies for muscular dystrophies), and (i) addressing ethics and communications issues (address the ethical and safety dimension for the development of therapies, as well as the need for a clear strategy of communication to lay and scientific audiences of the work of the network).

C. NEEDED RESEARCH RESOURCES

Preclinical Research Infrastructure

- *Research Objective 1:* Facilitate research (discovery, validation, and dissemination) of the biochemical pathways involved in muscular dystrophy (Medium Term; Intermediate Risk).

Several of the muscular dystrophies are in a relatively unique position of having substantial biochemical pathway data already known, and many of the downstream consequences of biochemical defects in these pathways are evident. Facilitation of studies by collaborative teams of biochemists, molecular geneticists, and bioinformaticians are encouraged to develop public resources that assemble all domain knowledge of biochemical pathways in the muscular dystrophies. The emphasis should be on characterization of key pathway components, with particular attention to muscle-specific members of ubiquitous pathways. The subgroup considered this as part of a larger-vision “road map” for the muscular dystrophies that could not only advance mechanistic understanding of the muscular dystrophies, but also identify viable drug targets.

- *Research Objective 2:* Establish standardized endpoints for preclinical trials in both mouse models, and the dog model, and ensure that facilities are available that enable testing of drugs and other therapeutic approaches (Long Term; Low Risk).

Animal trials should be designed with validated and standardized endpoints, such that the Food and Drug Administration would subsequently view the data as robust when considering Investigational New Drug applications for human clinical trials. Functional endpoints with significance for the target patient populations (i.e., reducing the clinical burden of disease) are most appropriate as they have the greatest likelihood of influencing clinical practice.

- *Research Objective 3:* Create a mechanism to maintain mouse models of muscular dystrophy at approved vendors in a live state, available for easy and rapid importation into academic colonies (Medium Term; Low Risk).

Mouse models are essential for mechanistic studies and translational research, particularly for medium-throughput screening and efficacy testing of potential therapeutics. For any new drug that shows promise, extensive mouse model testing bolsters Investigational New Drug

applications to the Food and Drug Administration. Despite the importance of mouse models, it can be difficult to determine how to obtain certain models; moreover there are few, if any, standard methods of testing mice and few facilities exist with standardized protocols to test drugs or other therapeutic approaches in mouse models. Existing strains and places to obtain animals or breeding pairs, and sites for functionally testing both disease progression and therapeutic efficacy, should be surveyed and tracked on an accessible website. The adequacy of existing facilities should be evaluated to ensure that they are sufficient to meet demands.

- *Research Objective 4:* Develop optimized models for mechanistic studies of specific muscular dystrophies, including models appropriate for therapeutic development screens (Medium Term; High Risk).

Efforts should be undertaken to develop mouse models for those muscular dystrophies for which such models do not currently exist, and to improve the existing mouse models for Duchenne muscular dystrophy. Expansion of animal testing capabilities for screening and evaluating therapeutic agents is also an important goal to facilitate the preclinical testing of novel agents. While animal-based testing cannot truly be high-throughput, efforts to generate strategies to allow meaningful indicators of therapeutic response in large numbers of animals in parallel should be encouraged. These initial screens can then be augmented with subsequent, detailed evaluations to probe mode of action and impact on pathogenesis.

- *Research Objective 5:* Encourage the development of cell-based assays that target aspects of pathogenesis and pathophysiology in the muscular dystrophies, to enable high throughput drug screening (Medium Term; High Risk).

There is desperate need to develop new therapeutics for the muscular dystrophies. Two types of therapeutic interventions can be imagined. The first is correction of the disease in instances where there is simply a missing protein. This has been the major focus of the field, and currently is envisioned with either viral gene therapy or cell-based therapy. Such approaches face major technical hurdles before implementation, and therefore are long-term goals for the field. For a number of diseases that result from more complicated mechanisms than a mutation in a single gene (e.g., facioscapulohumeral muscular dystrophy and myotonic dystrophy), such approaches may or may not be applicable. For these cases, as well as for developing approaches with a broad impact on a number of muscular dystrophies, there is need for a second type of therapeutic development. Namely, efforts need to be directed toward discovering agents (especially small molecules, RNAi, or shRNA) that can be easily delivered and that will target and slow aspects of the muscle pathogenesis in various forms of muscular dystrophy.

Even with candidate small molecule therapeutics in hand, there are substantial barriers to translation of animal studies to clinical trials, including adequate funding sources for medicinal chemistry facilities and personnel to provide chemical optimization, synthesis and characterization of small molecules based on the compounds with activity in animal models, lack of in-vitro assays to do high- or low-throughput studies of compounds and a less than optimal animal model for more advanced testing. In the clinical arena, there are well established clinical trial networks with very accurate and well defined strength and functional outcome measures where these drugs could be easily tested in a Phase II setting. These networks are also characterizing disease specific quality of life instruments and more advanced functional outcome measures to use in Phase II trials. In all cases, increasing industry involvement is essential, especially for the intermediate efforts (medicinal chemistry) and large Phase III trials for moving the therapies forward and ultimately into clinical practice.

A major obstacle to high-throughput screening is the inability of cultured cells from either humans or animals with muscular dystrophy to recapitulate the disease phenotype. While there are clearly instances, such as immune involvement, which cannot be mimicked in culture systems, it should be possible to develop models of cellular injury that would allow meaningful high-throughput screens.

Finally, there is a real need to increase industry involvement in the development of therapeutics for all forms of muscular dystrophy. Over the past five years, the first interest on the part of industry in the development of therapeutics for muscular dystrophy has emerged. There are at least two models for industry involvement in a rare disease such as muscular dystrophy. In one model, a drug may have wide application within the human population, as well as the possibility of affecting one or more of the muscular dystrophies. In this case, the orphan disease status of the muscular dystrophy, combined with the rapid progression of the disease, creates a fast track to approval of the therapy. Thus, even a large pharmaceutical company may become interested in an orphan disease. In the other model of therapies directed solely at one or more muscular dystrophies, the niche market, orphan disease status and lack of interest on the part of large pharmaceutical companies, make muscular dystrophy attractive for therapeutic development by small biotechnology companies. Nonetheless, increased industry involvement should be encouraged by specific funding programs that target therapeutic development for the muscular dystrophies. These programs should be designed to support all phases of therapeutic development and early phases of clinical trials. There should also be specific programs to promote increased academic-industry partnerships.

Clinical Research and Trial Infrastructure

- *Research Objective 6:* Establish a focus panel for molecular diagnostics of the muscular dystrophies, with the charge of developing consensus standards and approaches for molecular testing, screening, interpretation of results, and genetic counseling (Long Term; Low Risk).

A major challenge of this focus panel will be to identify methods by which such consensus standards can be delivered homogeneously throughout the United States. The Ataxia Diagnostics Group is a potential model for this effort.

Consensus on testing for Duchenne muscular dystrophy. Currently, many patients are diagnosed by either immunostaining of muscle biopsy or deletion testing, but the identification of point mutations or duplications is not uniform. Complete genetic characterization is becoming increasingly important for numerous reasons. First of all, accurate genetic counseling and availability for treatment is dependent upon an accurate genetic diagnosis. In addition, the prescription of effective treatments could quite possibly be tailored to specific genetic causes, a complete genetic characterization is increasingly important for all patients.

Consensus protocol for limb girdle muscular dystrophies. Currently, limb girdle muscular dystrophy is often diagnosed clinically with non-specific biopsy results and variable immunostaining characterization. Recently, available genetic screens for many common mutations have been helpful in better defining some patients, but complete genetic testing of all patients is unavailable and unaffordable. It would be useful to provide a protocol to assist clinicians as well as muscle biopsy directors with the task of characterizing apparent limb girdle muscular dystrophy patients. This would be invaluable in improving the frequency and accuracy of diagnosis.

Consensus protocol for facioscapulohumeral muscular dystrophy. The last several years have seen a much improved diagnostic protocol for facioscapulohumeral muscular dystrophy. Because of the complexity of the algorithm and mechanics of testing, there is a need for improved documentation, training, and education for professionals (physicians, neurologists, genetic counselors) on interpretation of genetic testing for facioscapulohumeral muscular dystrophy.

Improved education and interpretation of results. Many clinicians order testing indiscriminately, thereby increasing the cost of testing without increasing the yield. To the extent possible, generation of protocols or algorithms that focus and streamline testing would improve the diagnosis and decrease cost. Development of a publicly accessible website, or use of existing websites, to host this type of information would be valuable to practicing clinicians. Web-based information to assist clinicians in interpretation of results is also needed, and possibly access to individuals knowledgeable about the current status of testing in the various forms of muscular dystrophy.

Standardization of testing between laboratories. Many laboratories are offering comparable testing, without a mechanism for standardization of test results. An organization of diagnostic laboratories that facilitated development of consensus standards and approaches would improve reliability of testing, improve the diagnostic infrastructure, and would be an important adjunct to ongoing and upcoming clinical trials and research.

Improved prenatal, pre-implantation and carrier testing. One important means of reducing the number of affected muscular dystrophy patients is through accurate and meaningful genetic counseling. Effective counseling is dependent upon the availability of testing for genetic status at multiple levels, including parental, embryonic and fetal. This dictates a high level of testing accuracy, but also clinically focused laboratories that can provide reliable information quickly.

- *Research Objective 7:* Identify, develop, and encourage the use of standardized instruments to measure quality of life, cognitive, and central nervous system function using existing databases, and potentially develop new common element databases to extend research capabilities (Medium Term; Intermediate Risk).

A consensus should be reached on the identity of currently available quality of life instruments that are validated and appropriate for use in assessing muscular dystrophy patients and unaffected family members (including children—infants to teenagers—with Duchenne muscular dystrophy, limb girdle muscular dystrophy, myotonic muscular dystrophy, and facioscapulohumeral muscular dystrophy at different stages of handicap, as well as adults with these disorders). Steps should be taken to develop and validate new instruments and facilitate the training of those administering these new instruments. This process will require cooperation and collaboration between different disciplines and between different agencies within Federal and State governments as well as with private organizations.

Individual investigator-initiated and collaborative research efforts should be directed toward assessment of existing instruments with the capacity to evaluate cognitive and behavioral function in muscular dystrophy patient populations. This assessment is needed in order to determine the type and extent of infrastructure that will be required to reach long-term goals. Data mining/analysis of those test instruments being utilized in currently studied patient populations should be used to identify additional infrastructure needs. These databases include National Institutes of Health- and Muscular Dystrophy Association-funded clinical investigations

of the different muscular dystrophies (e.g., National Registry of Myotonic Dystrophy and Facioscapulohumeral Muscular Dystrophy Patients and Family Members, CINRG-University of California at Davis study, the Utah Dystrophin Project, and the Ohio State University limb girdle muscular dystrophy natural history study), Centers for Disease Control and Prevention-funded studies of Duchenne/Becker muscular dystrophy (MD STARnet), and the neuromuscular clinical patient populations affiliated with the National Institutes of Health-supported Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Centers.

Existing instruments with the capacity to assess cognitive/neurobehavioral/other central nervous system functions (e.g., sleep and neuroendocrine regulation) should be identified and evaluated as potential tools for studies of patients with the muscular dystrophies. Such tools should take into account children—infants to teenagers—with Duchenne muscular dystrophy, limb girdle muscular dystrophy, myotonic muscular dystrophy (DM1), and facioscapulohumeral muscular dystrophy at different stages of handicap as well as adults with these disorders. Research should be initiated to develop and validate new instruments and facilitate the training of those administering them for cognitive and behavioral assessment. This process will require cooperation and collaboration between different disciplines and between different agencies within Federal and State governments as well as with private organizations.

It is vital to develop research infrastructure (e.g., patient database networks) to nurture investigations of specific groups of patients with muscular dystrophy with higher risk of cognitive and central nervous system dysfunction (Duchenne muscular dystrophy; congenital muscular dystrophy; congenital myotonic dystrophy; and adult onset myotonic dystrophy, including both DM1 and DM2).

- *Research Objective 8:* Monitor, coordinate, and communicate the rehabilitation and educational assessment activities of the various Federal agencies, voluntary, and patient advocacy groups (Long Term; Low Risk).

Additional research designed to identify the high priority rehabilitation needs of patients with the different forms of muscular dystrophy (at critical stages in each) is necessary as a pre-requisite to determine the need for additional resources and infrastructure to investigate rehabilitation care and treatment of associated medical problems. Mining of currently available databases, prospective studies using existing and new patient databases, studies using patient reported outcomes of care of associated medical problems (PROMIS network), and studies that include partnerships with large patient populations, such as, the muscular dystrophy clinics, National Institutes of Health- and Muscular Dystrophy Association-funded clinical investigations of the different muscular dystrophies, Centers for Disease Control and Prevention-funded studies of Duchenne/Becker muscular dystrophy, and the neuromuscular clinical patient populations affiliated with the National Institutes of Health-supported Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Centers are all examples of approaches to identify the need for additional resources and infrastructure. Patients, health care providers, social services, health care economists, government agencies, and private industry, as well as researchers, are groups that should be encouraged to apply for and obtain grants or other funding for these studies. Compatible computer networking is needed to accomplish these investigations.

Studies should identify the highest priority medical problems that occur in the different types of muscular dystrophy and create training, funding, and research opportunities for individual grants and for partnering with various Government and non-government groups to pursue studies to evaluate and treat the associated medical problems. The evaluation of problems that occur at

specific stages of each of the muscular dystrophies will be a necessary part of achieving this goal.

Communication and Education

- *Research Objective 9:* Design and implement a web site that provides information and links to all existing resources in both the USA and internationally (Short Term; Low Risk).

Collaborative team approaches are becoming necessary in order to translate basic mechanistic findings into novel disease treatments and to ensure the coordinated management of what are frequently multi-system diseases. As these interdisciplinary efforts in the muscular dystrophies expand, one key rate-limiting factor, access to a variety of types of information, can be eliminated rather easily through the establishment of web-based catalogs of available resources. Genetic Alliance's CDC-sponsored resource center will provide an organized portal for resources related to Duchenne muscular dystrophy in the United States.

Both formal and informal tissue/cell line repositories exist for muscular dystrophy patients, but these are difficult to find, and it is even more difficult to determine what can be obtained from each. Efforts to develop a catalog of resources available from known or potential patient repositories should include a brief description of the resource, the procedures for access to samples, any fee structure, and any requirements for acknowledgement or co-authorship.

Gene mutation databases are essential to studies of disease mechanisms, development of diagnostic/screening tools, and initiation of molecular therapy-based clinical trials. The mutation database at Leiden University Medical Center (http://www.dmd.nl/) is among the most developed mutation databases in any disease. Other muscular dystrophy mutation databases are listed in the chapter on Diagnosis and Screening in Muscular Dystrophy. The mRNA expression profiling databases and public access tools for neuromuscular disease from Children's National Medical Center are highly evolved. However, both proteomics and development of sophisticated biochemical pathway tools have lagged behind similar efforts in other disease systems. Both bioinformatics as applied to biochemical pathway information (e.g. Ingenuity Inc type interfaces), and proteomics efforts, need to be encouraged and fostered in the muscular dystrophies.

Antibodies are important research tools, and are now used extensively for molecular diagnostics. Dr. Glenn Morris has created an impressive battery of muscular dystrophy-relevant monoclonal antibodies, and has received funding from the Muscular Dystrophy Association TRAC committee to distribute these at cost. Antibodies from Dr. Morris, and other laboratories willing to provide aliquots, should be listed on a web site. Muscular dystrophy-relevant antibodies, including protocols for receiving aliquots, the size aliquots that can/will be sent, and any costs associated with these should be listed as well.

- *Research Objective 10:* Establish a USA equivalent of the European Neuromuscular Centre's disease focus meetings to link to and communicate with European and other international networks or groups (Short Term; Low Risk).

Small, focused research meetings, with a clear agenda and requirement for specific outcomes, can be invaluable in establishing collaborations, reaching consensus, and moving a field forward. The standard for the muscular dystrophy field has been the European Neuromuscular Centre's continuing series of meetings that has had proceedings published in the journal *Neuromuscular Disorders*. A complementary series should be established in the USA, focusing

on the muscular dystrophies and with similar participant numbers and deliverables. USA—European partnerships should be encouraged; a means for USA investigator participation in the European Neuromuscular Centre's meetings, and corresponding European investigator participation in USA meetings, should also be provided.

- *Research Objective 11:* Increase the number and scientific breadth of basic scientists and clinicians involved in translational research in the muscular dystrophies (Long Term; Intermediate Risk).

While substantial progress has been made in the mechanistic understanding of many types of muscular dystrophy, few of these findings have translated into potential therapies for the disease. Frequently, academic investigators lack the expertise to undertake the steps necessary for therapeutic development up to Food and Drug Administration approval of an Investigational New Drug application. Training and research support vehicles should be put into place to ensure that major mechanistic discoveries are fully evaluated to determine potential therapeutic targets. Due to the nature of translational research, there is also a significant need to increase the breadth of disciplines from which these researchers are coming to include, developmental biology, systems physiology, the range of medical specialties (neurology, cardiology, pulmonology, orthopedics, ophthalmology, and rehabilitation medicine), nursing, social work, and psychology.

- *Research Objective 12:* Provide a publicly accessible listing of available training grants and resources so that opportunities for physicians and scientists are transparent (Short Term; Low Risk).

There remains a vital need to train and recruit new clinical and basic scientists to pursue research in the muscular dystrophies. Efforts should be made to enhance the visibility and availability of information about existing opportunities for training and education of research fellows. To improve the multi-disciplinary approach to the muscular dystrophies, there should be a broadening of the base of disciplines receiving training to include not only the medical and basic biological sciences but also the social sciences and medical economics. To this end, new fellowship opportunities should be created in nursing, physical therapy/occupational therapy, social work, clinical psychology, epidemiology, and health care management/economics in order to facilitate research in areas such as: living with muscular dystrophy, measuring/reducing the clinical burden of muscular dystrophy, and identifying optimal endpoints for disease manifestations of muscular dystrophy. These new grant opportunities and approaches should be developed using existing infrastructure, such as the Senator Paul D. Wellstone Muscular Dystrophy Collaborative Research Center Network, the Muscular Dystrophy Association-initiated PROD network, and the Centers for Disease Control and Prevention MD STARnet project, to enhance the education of patients and care providers on the subject of the muscular dystrophies. It is imperative to provide funding for grants, conferences, and communications networks that will help establish guidelines for care of patients with different forms of muscular dystrophy. This should include the establishment of networks of clinical centers, research groups, patient groups, professional societies, and other stakeholders (Government and private foundations) to maximize the use of existing resources and create optimal platforms for communication through computers and other means.

- *Research Objective 13:* Stimulate international collaborations and infrastructure sharing to ensure that opportunities are exploited and resources are used to maximum advantage, particularly in cases of novel opportunity or for the rare and/or understudied muscular dystrophies (Short Term; Intermediate Risk).

There is a critical need for better understanding of the mechanisms of some of the under-studied muscular dystrophies. This current deficiency in knowledge can have various causes; the most important ones being a complex etiology and low prevalence. In both situations the critical mass to swiftly proceed is lacking due to insufficient local multidisciplinary expertise, access to material etc. Pooling of scientific and patient resources is an attractive and cost-effective solution. Collaborations and sharing of resources among the international research groups focusing on these diseases will maximize the opportunities for discovery and will accelerate the accumulation of knowledge necessary for the development of effective treatment strategies.

D. MATRIX OF RESEARCH OBJECTIVES IN RESEARCH INFRASTRUCTURE NEEDS FOR MUSCULAR DYSTROPHY

	Short Term (0-3 years)	Medium Term (4-6 years)	Long term (7-10 years)
High Risk		• Develop optimized models for mechanistic studies of specific muscular dystrophies, including models appropriate for therapeutic development screens • Encourage the development of cell-based assays that target aspects of pathogenesis and pathophysiology in the muscular dystrophies, to enable high throughput drug screening	
Intermediate Risk	• Stimulate international collaborations and infrastructure sharing to ensure that opportunities are exploited and resources are used to maximum advantage, particularly in cases of novel opportunity or for the rare and/or understudied muscular dystrophies	Facilitate research (discovery, validation, and dissemination) of the biochemical pathways involved in muscular dystrophy • Identify, develop, and encourage the use of standardized instruments to measure quality of life, cognitive, and central nervous system function using existing databases and potentially develop new common element databases to extend research capabilities	• Increase the number and scientific breadth of basic scientists and clinicians involved in translational research in the muscular dystrophies
Low Risk	• Design and implement a web site that provides information and links to all existing resources in both the USA and internationally • Establish a USA equivalent of the European Neuromuscular Centre's disease focus meetings to link to and communicate with European and other international networks or groups • Provide a publicly accessible listing of available training grants and resources so that opportunities for physicians and scientists are transparent	• Create a mechanism to maintain mouse models of muscular dystrophy at approved vendors in a live state, available for easy and rapid importation into academic colonies	• Establish standardized endpoints for preclinical trials in both mouse models, and the dog model, and ensure that facilities are available that enable testing of drugs and other therapeutic approaches • Establish a focus panel for molecular diagnostics of the muscular dystrophies, with the charge of developing consensus standards and approaches for molecular testing, screening, interpretation of results, and genetic counseling • Monitor, coordinate, and communicate the rehabilitation and educational assessment activities of the various Federal agencies, voluntary, and patient advocacy groups

ACTION PLAN IMPLEMENTATION, TRACKING, AND ASSESSMENT

The Action Plan for the Muscular Dystrophies was developed with significant input from the leading researchers and clinicians in the field, and subsequently reviewed and approved by the MDCC. The goal was to identify Research Objectives that have the greatest priority and potential for success in reducing the clinical burden of disease. This Action Plan should not be perceived as a final, static document, but instead as an initial set of goals that will be implemented, tracked, and periodically re-assessed to ensure the best possible coordination of resources from the public and private sectors for detecting, diagnosing, treating, and preventing the muscular dystrophies.

A. PLAN IMPLEMENTATION

The Action Plan for the Muscular Dystrophies was developed by the MDCC SWG and the MDCC as a resource document for the entire muscular dystrophy research and education community. The Research Objectives identified here touch upon all aspects of the muscular dystrophies, from identification of pathogenic mechanisms to clinical management of patients with these diseases. While the NIH has played a leading role in the basic science discoveries that will help drive therapeutic development, given the mission of, and expertise and resources present at, the Federal agencies that are represented on the MDCC, many aspects of the Action Plan are most appropriately addressed by agencies besides the NIH. In addition, many of the activities that will be initiated as a result of the Action Plan will be facilitated by the leadership of the muscular dystrophy patient advocacy groups, particularly given their wide access to, and deep understanding of, the muscular dystrophy patient and family community. Indeed, many Federal agencies and advocacy organizations are already engaged in the types of activities that were identified as priorities by the MDCC SWG. Future activities may include the continuation and/or expansion of existing efforts, as well as the development of new initiatives by MDCC member agencies and organizations.

Many of the activities that will emerge from the Research Objectives identified in the Action Plan will be, by necessity, collaborative in nature. Because of the breadth of available knowledge and research tools, the multi-system complexity of disease mechanisms, and the need for rapidly translating basic findings into effective therapies, the research teams of the future must be comprised of multidisciplinary, interactive groups. Similarly, due to the scope of the emerging opportunities in the muscular dystrophies, and the financial resources that these require, there is also a real need for active collaborations between the agencies and organizations that invest in the research. The MDCC should play an important role in identifying and implementing collaborations among the various Federal agencies and facilitating collaborations between public agencies and voluntary patient advocacy groups in order to ensure the continuation of breakthroughs in reducing the clinical burden of muscular dystrophy. Working through its partners on the MDCC, the NIH will encourage and facilitate collaborative activities where they represent the best approach to achieving specific Research Objectives.

The Action Plan for the Muscular Dystrophies will also serve as a basis for communicating scientific priorities to the research community. Since its content reflects the expertise of the scientific leaders in the field, the Action Plan itself may serve as a stimulus for the design of basic and clinical research grant applications. The Action Plan will be publicized and made available on the MDCC website, and shared with students and postdoctoral fellows working in

the muscular dystrophy field, current and prospective grantees, and members of the appropriate Federal advisory committees and initial review groups.

Taken together, success in achieving the Research Objectives of the Action Plan for the Muscular Dystrophies will require coordination among the public agencies and private organizations that comprise the MDCC to ensure efficient use of scarce manpower and fiscal resources and avoidance of unnecessary duplication of efforts.

B. PLAN TRACKING

The NIH, together with its partner agencies and organizations in the MDCC, will ensure that progress relevant to this Action Plan is tracked and made publicly available. Future accomplishments and initiatives by all MDCC member agencies and organizations relative to the Action Plan will be tracked by means of the MDCC public website (http://www.ninds.nih.gov/find_people/groups/mdcc/index.htm). A record of key publications, conferences and workshops, and initiatives that are relevant to specific Research Objectives will be maintained on the MDCC website as well. This ensures that progress in achieving the Research Objectives of the Action Plan can be viewed by Governmental officials, advocacy groups, physicians, basic and clinical researchers, care providers, and patients and their families. MDCC members will present annual reports on activities relative to the Action Plan at the regularly scheduled MDCC meetings.

C. PLAN ASSESSMENT

The Action Plan for the Muscular Dystrophies is not meant to be a static document, but rather a snapshot at this point in time of the judgment of leading investigators in the field as to where current application of effort and resources can best address the most compelling opportunities. The Action Plan will require active tracking and assessment if it is to serve the purpose of stimulating research in the field and guiding activities of the MDCC. The 76 Research Objectives identified in the Action Plan will be used as guideposts for assessing progress and the Action Plan will be periodically reviewed and revised. Any revisions to the Action Plan will be included in the MDCC biennial report to Congress, as required in the MD-CARE Act. As appropriate, the MDCC may reconvene a scientific working group to assist in Action Plan assessment and revision. Reporting on progress made toward the Research Objectives will occur at future MDCC meetings and will be tracked on the MDCC website, providing a public record of progress toward the goal of improving the detection, diagnosis, treatment, and prevention of the muscular dystrophies.