Duchenne muscular dystrophy

Jeffrey S. Chamberlain

University of Michigan Medical School, Ann Arbor, Michigan, USA

Progress in understanding the role of dystrophin raises promising hopes for a treatment for Duchenne muscular dystrophy. In addition, great improvements have been made in the ability to diagnose this disease using simple molecular methods.

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Introduction

More than 4 years have passed since the gene responsible for Duchenne/Becker muscular dystrophy (DMD/BMD) was identified. Progress in understanding this gene and its role in disease has followed at a breathtaking pace, and the rapidly emerging information has been summarized in at least three reviews in the past year alone [1–3]. This article is intended to highlight only the most significant advances made in the past year, and focuses on three specific areas: developing efficient and cost-effective methods for diagnosis and carrier detection of DMD; understanding the role of the DMD gene product, dystrophin; and developing approaches to a treatment or cure for the disease. In the absence of a treatment, disease diagnosis and genetic counseling remain the most concrete example of how recent progress is of benefit to patients. The understanding of how defects in the dystrophin gene lead to various manifestations of the disease is critical for an understanding of the role played by dystrophin in various tissue types, which, in turn, is important for an intelligent approach to developing and implementing treatments for DMD.

Diagnosis of DMD

Deletion mutations account for the majority of all cases of DMD, but the enormous size of the locus ($> 2.3 \times 10^6$ bp) has prevented any simple assay of the integrity of the gene or even a detailed understanding of the gene structure. Den Dunnen *et al.* [4•] have reported a detailed analysis that has refined knowledge of the gene structure, particularly in the deletion-prone central region. Boyce *et al.* have localized and characterized the extreme 5′ end of the locus, corresponding to the promoter region used predominantly in brain tissue [5•]. This additional knowledge of the gene structure is critical for DNA-based diagnosis of DMD. Two groups have reported refinements of tests for the detection of deletions using the polymerase chain reaction (PCR) [6•,7•]. These assays can be used to scan for deletions in the gene, enabling direct detection of the majority of cases with a simple assay requiring one day to perform. Partial gene duplication mutations have been shown to account for an additional 5–10% of DMD cases, and knowledge of the gene structure has facilitated analysis of the effects of these mutations and their likely origin [8•].

Approximately 30% of DMD cases result from subtle mutations not readily detectable with current methodologies, and family studies involving these cases are performed using linkage analysis of polymorphic loci that both lie within and flank the dystrophin gene. PCR promises to greatly facilitate these assays, and is soon likely to be the only technique needed for the diagnosis of the vast majority of cases. Two groups have reported PCR assays that enable efficient haplotyping using highly informative microsatellite repeat markers at either end of the locus [9•,10•]. The dystrophin gene has been shown to be a very recombinagenic locus, which increases the possibility of making an erroneous diagnosis based upon linkage analysis [11•]. The identification of additional microsatellite repeats throughout the central region of the gene now makes it possible to track these recombination events and to perform accurate haplotyping on most DMD families (J Chamberlain, P Clemens, CT Caskey, unpublished data).

Two groups have reported an exciting alternative to DNA-based diagnosis, whereby the dystrophin mRNA is amplified to allow assessment of its size, again using PCR [12•,13•]. These assays have the advantage of being able to detect most structural rearrangements of the gene in affected males as well as in many carrier females, but although they require nothing more than a blood sample, these tests are not as simple to perform as the DNA assays. Finally, tests that examine the integrity of the dystrophin protein directly have been refined, and antibodies can now be used to identify the size of a defective protein species produced in dystrophic muscle [14•]. This information can also be used to understand the severity of the disease in relation to particular mutations.

Abbreviations

BMD—Becker muscular dystrophy; **DMD**—Duchenne muscular dystrophy; **PCR**—polymerase chain reaction.

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The function of dystrophin

Perhaps the most exciting development of the past year was the increased understanding of the role dystrophin plays in normal muscle tissue. Ervasti *et al.* reported the identification of a protein complex associated with dystrophin [15••]. These data indicate that dystrophin may play an important role in regulating the activity or function of specific membrane proteins. Although it is not yet clear what this function may be, two reports of altered calcium channels in dystrophic muscle strongly implicate a role for dystrophin in calcium transport and/or homeostasis [16••,17••]. It will be exciting to learn whether the membrane proteins identified by Ervasti *et al.* [15••] either regulate or act as calcium channels. It has been thought by many researchers that dystrophin plays a structural or mechanical role in reinforcing the sarcolemma; now it seems likely that the protein plays a dual role, regulating the activity of membrane proteins as well as acting as a structural component of the membrane. Direct evidence for a structural role comes not only from previous antibody experiments but also from a recent report that demonstrated that dystrophic muscle cells are unusually sensitive to osmotic shock [18•]. A detailed report on the likely structure of dystrophin supports the concept of a structural role [19•], and has implications for the effects of internal truncations of the protein that lead to mild phenotypes. A patient reported by England *et al.* [20•] was found to lack almost 50% of the dystrophin amino acid sequence, yet displayed a surprisingly mild phenotype. The ability to correlate domains of the protein that are essential or non-essential for proper functioning of dystrophin may facilitate the design of vectors for gene therapy (see below). Finally, whereas studies of the role of dystrophin have focused mainly upon skeletal muscle, Lidov *et al.* [21••] identified the location of at least one isoform of dystrophin within brain tissue. The observed brain localization implies that dystrophin plays a unique role in nerve cells, and it will be important to determine which proteins are associated with dystrophin in non-muscle tissues.

Therapy for DMD

Despite recent progress on many fronts, there is still no treatment or cure for DMD. At least two areas of study appear promising, and both aim at directly replacing the synthesis of dystrophin in affected tissues. Myoblast transplantation therapy has been extensively discussed as a method to provide skeletal muscle with normal dystrophin, and was the focus of an international conference in 1989 that brought together many leading muscle researchers. The proceedings of this meeting have been published in a book that addresses the problems, hopes and challenges facing investigators hoping to replace the myogenic stem cell population with donor myoblasts in dystrophic skeletal muscle [22•]. Although these experiments have been pioneered in mouse model systems, the urgency of finding a cure has prompted several groups to launch human trials with minimal animal experimentation. To date only preliminary results have been reported [23•], and it remains to be demonstrated whether this technique is feasible.

An even more untested approach involves gene replacement therapy. As the dystrophin gene is of such enormous size, most efforts are focused upon developing methods to deliver dystrophin 'minigenes' to affected tissues. Lee *et al.* [24••] have reported the construction and expression of a dystrophin minigene vector that was made using a full length cDNA clone, and that produces normal dystrophin when transferred into a variety of cell types. Directing the expression of such a vector will require appropriate tissue-specific promoter elements, and Klamut *et al.* [25•] have identified and characterized the major dystrophin gene promoter, which is predominantly used in muscle. This work raises the possibility of using the endogenous dystrophin promoter for minigene expression in therapeutic trials. Development of methods by which minigenes could be delivered to patients will require further research, although a surprisingly simple method was reported by Wolff *et al.* [26••]. These authors showed that plasmid DNA injected directly into muscle could be incorporated into the tissue and expressed for at least several months' duration.

Although both myoblast and gene therapy are extremely experimental approaches for treating DMD, research in these areas is encouraging. Five years ago there was little reason to expect a quick breakthrough, and years of additional research certainly remain. In spite of this, however, continued study of the structure, function, and expression of the dystrophin gene, together with the development of approaches for dystrophin delivery to affected tissues, do raise hopes for the successful treatment of this devastating and relatively common genetic disease.

References and recommended reading

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- • of interest
- •• of outstanding interest

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JS Chamberlain, Department of Human Genetics, M4708 Medical Sciences II, Box 0618, University of Michigan Medical School, Ann Arbor, Michigan 48190-0618, USA.