INTRODUCTION

Muscular dystrophy is a class of inherited disorders characterized by progressive weakness and degeneration of skeletal muscles. Over 40 forms of muscular dystrophies have been identified, based on underlying genetic and molecular etiology, clinical manifestations and prognosis ***(Muir and Chamberlain, 2009).***

The current clinical classification of the muscular dystrophies is based mainly on the distribution of the dominant muscle weakness, however, several of the classical types have retained their eponymic designations; hence: Duchenne, Becker, Emery-Dreifuss, facioscapulohumeral, limb-girdle, occulopharyngeal, and distal types. To these are added myotonic dystrophy and a group of so called congenital muscular dystrophies, usually severe in degree. The extraordinary depth of information regarding the molecular nature of the dystrophies is one of the most gratifying developments of modern neuroscience ***(Kanagawa and Toda, 2006).***

The X-linked dystrophin gene is by far the largest of the 30,000 genes that encode proteins in the human genome: its 79 exons cover 2.6 million base pairs. This large size makes the

gene prone to rearrangement and recombination events that cause mutations. In most cases, the mutations are deletions of one or more exons (60%), however, duplications (6%), translocations and point mutations have also been found. In general, mutations that disrupt the reading frame of the dystrophin transcript and lead to prematurely aborted dystrophin synthesis cause Duchenne muscular dystrophy (DMD). DMD is the most frequent lethal heritable childhood disease: affecting 1 in every 3,500 live boys ***(White et al., 2002).***

Regardless of the type of dystrophy and stage of the disease, certain general principles must be considered. A well balanced diet with adequate fiber to overcome problems of constipation is essential, especially when individuals become immobile. Excessive weight gain may also become a problem. Prolonged periods of bed rest should be discouraged because they can accelerate weakening of the muscles. Though everyday activity within the individual's limits should be encouraged, strenuous exercise should be avoided (although supervised swimming is excellent exercise) ***(Emery, 1998).***

It is of major importance, in the treatment of almost all individuals with dystrophy, the preservation of respiratory function, which may be compromised by immobility and the

chest deformity that results from scoliosis. All respiratory infections must be treated thoroughly, as soon as they occur, with postural drainage and antibiotics. The surgical correction of scoliosis makes sitting easier and more comfortable and also helps to preserve lung function ***(Emery, 1998).***

It has been found that the ventricular remodeling of the cardic muscles may occur in males with Duchenne muscular dystrophy and Becker muscular dystrophy (BMD) with early diagnosis and treatment of cardiomyopathy ***(Jefferies et al., 2005).*** Children with DMD or BMD are usually treated early with an angiotensin converting enyme inhibitor and/or beta blocker. In cases of overt heart failure, other heart failure therapies including diuretics and digoxin are used as needed. Cardiac transplantation is offered to persons with severe dilated cardiomyopathy and BMD with limited or no clinical evidence of skeletal muscle disease ***(Towbin, 2003).***

Studies have shown that **prednisone** improves the strength and function of individuals with DMD. It is hypothesized that prednisone has a stabilizing effect on membranes and perhaps an anti-inflammatory effect. **Aminoglycoside** therapy has been suggested as an alternative to gene therapy. Indeed, in up to 15 percent of DMD patients, the dystrophin gene mutation is caused by a premature stop codon

created by a nonsense mutation; experimental data have demonstrated that Aminoglycoside treatment of cultured cells can suppress stop codons, which allows the insertion of alternative amino acids at the site of the mutated stop codon. In vivo Gentamicin therapy in the mdx mouse resulted in dystrophin expression of 10 to 20 percent of the levels detected in normal muscle, however the optimal treatment dose and mode of administration have not been determined ***(Barton-Davis et al., 1999).*** Recently, new Aminoglycosides, with no major side effects, called **PTC124** which belongs to a new class of small molecules that mimics at lower concentrations the read-through activity of gentamicin. The administration of PTC124 resulted in the production of full-length and functionally active dystrophin both in vitro and in mdx mice ***(Aurino and Nigro, 2006).***

Among the many programs of tissue engineering, gene therapy has been considered as the medicine of the 21st century. Despite the nearly universal belief that gene therapy will ultimately allow the treatment of currently incurable diseases and conditions, its potential remains largely unfulfilled. Only when a safe and effective gene delivery technology (vectors) has been proven in humans, can the full potential of gene therapy be realized ***(Rodino-Klapac et al., 2007).***

Since the initial characterization of the genetic defect for Duchenne muscular dystrophy, much effort has been expended in attempts to develop a therapy for this devastating childhood disease. Gene therapy was the obvious answer but, initially, the dystrophin gene and its product seemed too large and complex for this approach. However, the increasing knowledge of the organization of the gene and the role of dystrophin in muscle function has indicated ways to manipulate them both. The size of the dystrophin gene has been an important challenge for gene-therapy researchers. To replace a defective dystrophin gene, an artificial dystrophin DNA construct must be transferred into the nuclei of muscle cells, where it must be expressed and regulated appropriately ***(Warner et al., 2002).***

A series of large deletions that were evaluated in the central rod domain indicated that although the rod structure is indispensable, the number of repeats can be markedly reduced. A 6.2 kb mini-construct (mini-dystrophin) was found to be completely functional, transgenic mdx mice that carried this construct showed non-dystrophic muscle morphology and normal force generation. Further reduction is feasible, as shown by several micro-constructs (micro-dystrophin: 3.6–4.2 kb) that were highly effective in supporting almost normal muscle structure and function, at least in mice ***(Van Deutekom and Van Ommen, 2003).***

The relatively mild BMD phenotypes that are caused by some large deletions or nonsense mutations have also pointed to another possible gene-therapy strategy**: skipping an exon** during PRE-mRNA splicing to enlarge a DMD deletion so that it becomes its nearest in-frame BMD counterpart. The resulting transcripts are in-frame and allow the synthesis of internally deleted dystrophins that can, to a varying extent, lead to a milder phenotype. Such splicing anomalies are also thought to cause isolated clusters of 'revertant'(dystrophin-positive) myofibres in many DMD patients ***(Van Deutekom and Van Ommen, 2003).***

Myoblast-mediated delivery may be particularly advantageous for certain kinds of gene therapy, because the cells behave in a manner atypical to other types of cells ***(Blau and Springer, 1995).*** Myoblasts are muscle-building cells endogenous to the human body. Contained within the nucleus of each human myoblast is the normal genome with over 100,000 normal genes that determine cell normality and cell characteristics. Myoblast is the only somatic cell type in the body capable of natural cell fusion. Through this process, they insert their nuclei, and therefore all of the normal genes, into multinucleated myofibers of the host to effect genetic repair ***(Kapsa et al., 2003).***

Stem cells are defined as cells capable of self-renewal and of differentiating into multiple cell lineages ***(Sohn and Gussoni, 2004).*** Adult stem cells seem to have the capacity to "transdifferentiate" into cells of many different tissue types. The discovery of adult tissue specific stem cells, such as **haematopoietic stem** cells, which have the ability to "transdifferentiate" into other tissues, has generated much excitement among cell biologists and transplant clinicians. It opens new avenues for basic biological research by using stem cells from adults as an alternative to **stem cells from embryos.** It also carries important implications for the treatment of many liver, heart, and neurodegenerative diseases. Despite strong evidence that multipotent stem cells (stem cells with the potential to differentiate into several cell types) reside in many adult organs and can be manipulated in ways that may confer a therapeutic advantage, several questions remain to be addressed before the development of clinical applications ***(Kuehnle and Goodell, 2002).***

Since the isolation of stem cells from human embryos, the prospect of using such cells to generate cells and tissues for cell therapy has stimulated much scientific and public interest. The stem cells are obtained from the inner cell mass of the blastocyst. As numerous studies with their mouse counterparts have shown, the stem cells from human embryos are inherently

primitive, can proliferate indefinitely, and have the capacity to generate all cell types of the adult ***(Odorico et al., 2001).***

Bone marrow transplantation performed in the *mdx* mouse, as well as intravenous injection of normal muscle-derived stem cells, was successful in partially restoring dystrophin expression in the affected muscles. The intravenous route of administration of the stem cells allows a wider dissemination into the affected muscles than that accomplished with previous techniques (e.g., direct intramuscular injection of myoblasts). Further studies will be necessary to optimize these techniques in order to provide clinically useful levels of dystrophin, as well as to determine their applicability ' in humans. Nevertheless, stem cell therapy seems to be a promising therapeutic intervention for dystrophinopathies, assuming that skeletal muscle stem cells exist in humans. An alternative would be the use of bone marrow-derived stem cells ***(Wang et al., 2009).***

AIM OF THE WORK

- To review the molecular advances & recent genetics of muscular dystrophies.

- To highlight the recent molecular therapeutic interventions in patients with muscular dystrophies opening the way for their possible clinical applications in the near future.

Muscular Dystrophies and their Classification

Muscular dystrophies constitute a clinically and genetically heterogeneous group of skeletal muscle-wasting diseases ***(Kissel and Mendell, 1999)***. They describe a group of primary genetic disorders of the muscles that often have a distinctive and recognizable clinical phenotype, characterized by progressive weakness and degeneration of skeletal muscles with variable distribution and prognosis, accompanied by characteristic, but frequently not pathgnomonic, pathological features ***(Huang et al., 2008).*** The pattern of histological alterations associated with muscular dystrophy is diagnostic, a consequence of cyclic degeneration and regeneration of skeletal muscles (although cardiac and smooth muscle also may be involved). Muscle degeneration is typically progressive, with an apparent increase in severity as connective tissue accumulates and the intrinsic regenerative capacity of muscle becomes exhausted ***(Porter, 2000).***

Different types of muscular dystrophies

The most common form is the X-linked recessive **Duchenne muscular dystrophy (DMD),** named after Guillaume Benjamin Amand Duchenne, who described it in

1861. It commonly occurs at the age of 3-5 years and is presented clinically by Calf muscle hypertrophy, waddling gait and loss of ambulation at 7-12 years. Complications include severe cardiac and respiratory involvement, diagnosis is through a 5 to 100 fold elevated Creatine Kinase Level, Immunohistochemistry and Immunoblot techniques that reveal Dystrophin staining or blot absent in addition to genetic testing ***(Manzur and Muntoni, 2009).***

Unlike Duchenne, **Becker (BMD)** occurs above the age of 6 years and shows a more benign course with variable manifestations with later onset and slower progression also with severe cardiac and respiratory involvement. Investigations usually reveal Dystrophin staining, a reduced blot and a 50- to 100-fold elevated Creatine Kinase Level ***(Fadda et al., 1985).***

About 50 years after Duchenne, Batten published the first cases of **congenital muscular dystrophy (MDC)** ***(Schapira, 2008).*** The Congenital Muscular Dystrophy (CMD) is a group of muscular dystrophies that are symptomatic since birth. It shows an autosomal recessive pattern of inheritance. The disease usually progresses slowly with a tendacy to remain static for unspecified periods. The disease can also have periods of sudden and rapid worsening at any age. The frequency of CMD remains unclear. Fukuyama type of CMD which is

common in Japan has an incidence of 7-12 per 100,000 children ***(Shashikiran et al., 2003).*** Unlike patients with the DMD/BMD phenotype, patients with CMD present with weakness and dystrophic changes in the muscle biopsy at birth, but symptoms are less rapidly progressive than in DMD ***(Schapira, 2008).***

The term **limb girdle muscular dystrophy (LGMD)** was introduced in the middle of the 20th century, when it became obvious that there was an additional major group of non congenital muscular dystrophies that differed from both the X-linked DMD and BMD and from the autosomal-dominant facioscapulohumeral forms ***(Schapira, 2008).***

Limb girdle muscular dystrophy has changed from a wastebasket designation to an ever-expanding list of subtypes (18 subtypes), for which accurate molecular diagnoses are available. Age at onset usually ranges from early childhood to late adulthood ; however, the same gene defect can cause allelic forms of MDCs and LGMDs, as shown for the fukutin-related protein deficiencies ***(Bushby, 1995).***

The finding that mutations in nuclear envelope proteins also cause muscular dystrophies referred to as *nuclear* envelopathies or **Emery-Dreifuss muscular dystrophies**, came as a surprise when emerin, the protein shown to be mutated in X-linked Emery Dreifuss muscular dystrophy (EDMD1, X-

EDMD) ***(Bione et al., 1994)*** was also found to be an inner nuclear membrane protein. The importance of the nuclear envelope in neuromuscular disease was bolstered by the discoveries that mutations in the Lamin A/C (LMNA) gene can cause autosomal dominant Emery-Dreifuss muscular dystrophy (EDMD2), ***(Bonne et al., 1999)*** dilated cardiomyopathy with conduction defect (CMD1A), ***(Fatkin et al., 1999)*** limb-girdle muscular dystrophy type 1B (LGMD1B) and a variety of others ***(Muchir et al., 2000)***.

Facioscapulohumeral muscular dystrophy (FSHD), first described in 1885, is a frequent form of muscular dystrophy that has a distinctive clinical manifestation with autosomal dominant inheritance ***(Schapira, 2008).*** The gene underlying FSHD was mapped to chromosome 4q35 in 1992 ***(Wijmenga et al., 1992)*** and was shown to be closely linked to locus D4F104S1. Although D4F104S-associated deletions are closely associated with FSHD, the identity and location of the FSHD gene (or genes) still remain elusive ***(Schapira, 2008).***

Myotonic dystrophy (DM) is a dominantly inherited neurodegenerative disorder for which there is no cure or effective treatment. Investigation of DM pathogenesis has identified a novel disease mechanism that requires development of innovative therapeutic strategies. It is clear that DM is not

caused by expression of a mutant protein. Instead, DM is the first recognized example of an RNA-mediated disease. Expression of the mutated gene gives rise to an expanded repeat RNA that is directly toxic to cells. The mutant RNA is retained in the nucleus, forming ribonuclear inclusions in affected tissue. A primary consequence of RNA toxicity in DM is dysfunction of two classes of RNA binding proteins ***(Wheeler, 2008).***

Myotonic dystrophy type 1 (DM1) is the most common muscular dystrophy in adults, affecting approximately 1 in 7400. Inheritance is autosomal dominant and results from a CTG repeat expansion in the 3′ untranslated region of the DM protein kinase gene (DMPK) on chromosome 19q.2 As with several other types of muscular dystrophy, it is not limited to skeletal muscle. Instead it is a multisystemic disorder that includes myotonia, progressive weakness, muscle wasting, insulin resistance, cardiac conduction defects, neuropsychiatric symptoms, gonadal atrophy, and early cataracts ***(Wheeler, 2008).***

A second form of myotonic dystrophy, DM type 2 (DM2), was discovered. It results from an unstable expansion of a CCTG repeat in intron 1 of the zinc finger protein 9 gene (ZNF9) on chromosome 3q. DM2 shares the core features of DM1, including autosomal dominant inheritance, weakness,

myotonia, and multisystem involvement. In contrast to DM1, however, weakness and myotonia affect proximal leg muscles at onset, and muscle atrophy generally is less severe ***(Liquori et al., 2001).***

Oculopharyngeal muscular dystrophy (OPMD) is unusual among muscular dystrophies because of its manifestation in late adult life, typically in the sixth decade. Symptoms include progressive ptosis and dysphagia, followed by involvement of other cranial and limb muscles. OPMD is usually inherited as an autosomal dominant trait as a result of an expanded guanine-cytosine-guanine (GCG) repeat detectable in the poly A binding protein 2 gene on chromosome 14 ***(Brais et al., 1998; Brais et al., 1995).***

Distal myopathies are frequent in the Nordic countries and are being increasingly recognized elsewhere. Six different distal myopathy phenotypes have been identified, and there has been considerable progress in the understanding of the molecular pathophysiology underlying the distal myopathies ***(Penisson-Besnier, 2004).***Mutations in membrane-associated dysferlin cause two different distal phenotypes, allelic to LGMD2B, ***(Ueyama et al., 2002)*** whereas mutations in titin can cause either a distal myopathy (type Udd/Markesbery-Griggs) or LGMD2J, ***(Argov et al., 1997; Hackman et al., 2002)***