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Dystrophin and the two related genetic diseases, Duchenne and Becker muscular dystrophies

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Abstract

Mutations of the dystrophin *DMD* gene, essentially deletions of one or several exons, are the cause of two devastating and to date incurable diseases, Duchenne (DMD) and Becker (BMD) muscular dystrophies. Depending upon the preservation or not of the reading frame, dystrophin is completely absent in DMD, or present in either a mutated or a truncated form in BMD. DMD is a severe disease which leads to a premature death of the patients. Therapy approaches are evolving with the aim to transform the severe DMD in the BMD form of the disease by restoring the expression of a mutated or truncated dystrophin. These therapies are based on the assumption that BMD is a mild disease. However, this is not completely true as BMD patients are more or less severely affected and no molecular basis of this heterogeneity of the BMD form of the disease is yet understood. The aim of this review is to report for the correlation between dystrophin structures in BMD deletions in view of this heterogeneity and to emphasize that examining BMD patients in details is highly relevant to anticipate for DMD therapy effects.

KEYWORDS: Dystrophin; Becker muscular dystrophy; Duchenne muscular dystrophy; exon skipping; micro-dystrophin
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INTRODUCTION

Mutations of the dystrophin *DMD* gene are the cause of two devastating and to date incurable diseases, Duchenne (DMD) and Becker (BMD) muscular dystrophies [1]. *DMD* gene is the longest human gene with 2.4 Megabases of DNA representing ~1% of the chromosome X DNA [2, 3]. It is localized on the locus p21 of chromosome X and codes for the protein dystrophin. This large gene comprises 79 exons separated by very large introns which explain the giant size of the gene; the mRNA of the largest isoform of dystrophin is 14 kb which is only 0.6% of the total weight of the gene. The intron 44 is 170 kb on its own. Several promoters are active in a tissue-specificity manner and lead to the expression of full length or shorter dystrophins. The full length dystrophin is expressed in all striated skeletal, smooth and cardiac muscles. Shorter isoforms are expressed in brain cells and in retina.

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MUTATIONS OF THE DMD GENE

A high number of mutations of the *DMD* gene has been reported with ~65% being deletions of one or several exons, ~10% of duplications of exons and ~15% of single point mutations [4, 5]. Depending on the fact that mutations maintain or not of the reading frame, dystrophin will or not be present according to the Monaco rule [6]. In case of out-of-frame mutations, dystrophin is mostly deficient and this leads to the severe DMD disease. In case of in-frame mutations, dystrophin will be expressed as a mutated protein either with missense substitution or deletions or duplications of an internal part of the protein (Figure 1). These in-frame mutations mostly lead to the less severe BMD disease in accord with the Monaco rule but in certain cases, exceptions to the Monaco rule appear where a DMD phenotype with an in-frame mutation is observed. This is particularly prominent when mutations involve the N-terminal actin-binding-domain or the Cys-rich domain affecting the binding of dystrophin to F-actin or β-dystroglycan, respectively.

CLINICS, HISTOPATHOLOGY AND DYSTROPHIN IN DMD AND BMD

The first clinical signs of DMD are difficulties for young boys to walk and to climb stairs as early as 2-3 years of age. These boys

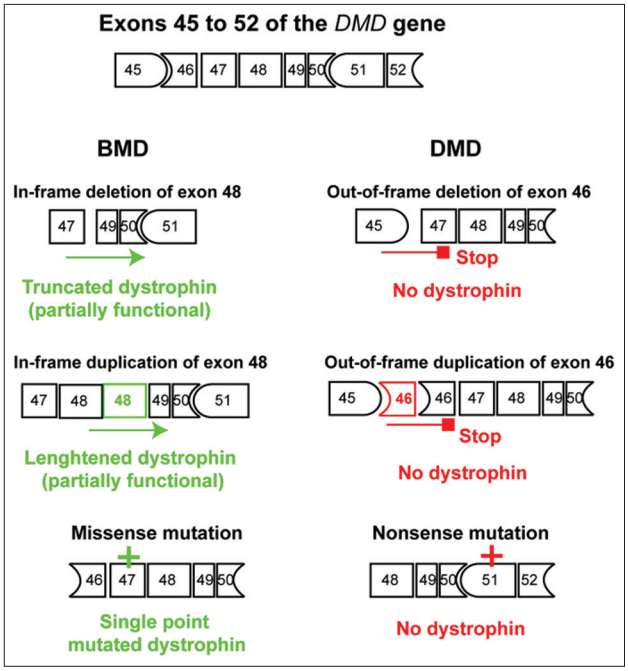


FIGURE 1. Examples of mutations of the DMD gene and their consequences on the production of dystrophin and the corresponding phenotypes. The boxes represent exons. Right faces of boxes indicate that the exon codes for an entire protein sequence based on 3-bases codons (examples: exons 47, 48, 49). Curved faces indicate that the exon does not code for an entire protein sequence but that either the first or the last bases need the preceding or following exon to code a full 3-bases codon (examples: exons 50, 51, 52). BMD for Becker muscular dystrophy; DMD for Duchenne muscular dystrophy.

never run and become wheelchair confined before their 12 years. Respiratory and cardiac impairments appear progressively and in most cases, patients do not survive after their 30 years (for a review focusing on clinics, see [7]). These clinical signs are due to a progressive muscle weakness involving all striated skeletal and cardiac muscles and are accompanied by highly elevated creatine kinase blood levels [8-10]. Similar clinical signs are observed for BMD patients but with very variable time course and severity. Some BMD patients are highly asymptomatic while some become wheelchair confined around 16 years of age. They could survive until very old ages or some of them die from an early heart failure [11, 12]. On the histological point of view, the DMD muscles show cycles of fiber necrosis and regeneration. However, regeneration is overtaken by fiber loss mechanisms and fibrosis and adipose tissue replacement are increased. Nuclei are in the normal muscle at the cell periphery and with the ongoing regeneration in DMD muscles, nuclei become centrally localized. All these features vary depending on the age of the DMD patients with fibrosis and fatty infiltration increasing with age and being highly variable in BMD. In DMD, electron microscopy reveals lesions of the plasma membrane [13] which indicates that the primary role of dystrophin is to maintain plasma membrane integrity.

Immunoblotting of dystrophin in DMD muscles reveals the total absence of dystrophin except in some revertant fibers. In BMD muscles, the expression of mutated dystrophin is observed but with highly variable extents from less than 10 % to as high as 75% of the full length expression of normal muscles [14, 15]. The direct correlation between dystrophin amount in BMD and clinical severity is not proven. However, it is recognized that measuring accurately the dystrophin level is highly challenging [16] partly explaining the lack of clear correlation between phenotype severity and dystrophin level. Other parameters could also be involved as there exist certain unexplained results which appear in contrast with the accepted rules [17].

DYSTROPHIN

Dystrophin consists of four major structural domains (Figure 2) [18, 19]. The N-terminal domain coded by exons 1 to 8 is mostly an actin-binding domain with two calponin-homology domains CH1 and CH2 [20-22]. The central part of dystrophin coded by exons 8 to 61 is made of 24 spectrin-like repeats interspaced by four hinges H1 to H4 and at the origin of the rod-shaped filament nature of dystrophin [23]. This long domain interacts with a high number of proteins among which they are the filamentous actin [24], intermediate filaments [25, 26] and microtubules [27, 28] and finally the muscular isoform of nitric oxide synthase (nNOS)[29-31] and PAR1-b [32]. In addition, this domain interacts with membrane phospholipids[33-35]. The third domain coded by exons 62 to 69 is the Cys-rich domain composed of a WW domain, two EF hands and a ZZ domain[36, 37]. This domain binds to the most important partner of dystrophin i.e. the membrane protein β-dystroglycan [38, 39] and to several other proteins such as plectin[40], ankyrin [41] and the intermediate filament protein synemin[25]. The C-terminal domain of dystrophin coded by exons 69 to 79 binds to the two cytoplasmic proteins syntrophin and dystrobrevin[42, 43].

Dystrophin is anchored to plasma membrane by phospholipids and β-dystroglycan which in turn interacts with extracellular matrix proteins (Figure 2). As such, dystrophin constitutes a major scaffolding protein of normal muscle which links cytoskeletal actin, microtubules and intermediate filaments to the extracellular matrix. This dystrophin scaffolding network is present at specific structures of the skeletal and heart muscle named costameres situated at the periphery of the fibers along with the transverse M- and Z-lines [44, 45]. Costameres are specifically involved in the lateral transmission of forces from the cytosol to the extracellular matrix by which they prevent plasma membrane ruptures during muscle contractions [41, 46-49].

DYSTROPHIN AND DMD AND BMD

The primary defects in both DMD and BMD are disruptions of this dystrophin scaffolding network by the absence of

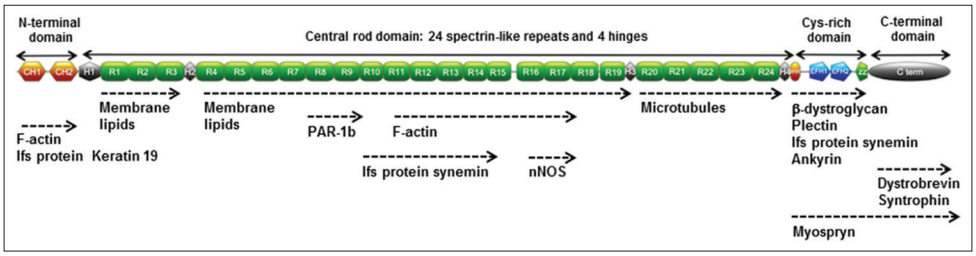


FIGURE 2. Dystrophin domains and partners. CH1 and CH2: calponin homology domains 1 and 2; H: unstructured regions called hinge; R: spectrin like repeat; WW: Tryptophan-rich domain; Z: ZZ domain; EFH: EF hand domains; PAR-1b: polarity regulating kinase 1b; nNOS: n-nitrous oxide synthase; Ifs: intermediate filaments.

dystrophin in DMD or due to mutations that ablate part of the protein as in BMD. The transmission of forces from the cytosol to the extracellular matrix at the costamere is impaired, leading to frequent ruptures of plasma membrane during contractions [13, 50]. This leads to leakage of cellular components such as creatine kinase from the interior of the muscle cells which explains the high plasma membrane creatine kinase levels in blood of children suffering from DMD. In addition, these ruptures of plasma membrane increase inside fluxes of calcium which subsequently activate calcium-dependent proteases [51].

It is remarkable that a primary defect i.e. the dystrophin deficit or mutation induces a progressive muscle impairment demonstrating that muscle is able to function without dystrophin but is not able to resist forces in the long time. That signifies that the therapy strategies could be very large from inducing dystrophin expression to compensatory therapies such as increasing blood flow with NO mimetic, increasing regeneration potential, surrogate protein expression... As well, the observation of the progression of the disease in BMD patients indicates that dystrophin could sustain partly its function even with mutations that ablate part of its central domain. In the reverse, missense mutations in the N-terminal ABD or in the Cys-rich domain could be accompanied by the DMD severe phenotype [4, 5]. Therefore, it is clear that certain parts of the molecule are more indispensable than others and this led to the idea that a therapy strategy could be to transform the severe DMD phenotype into a mild BMD phenotype, expressing mutation in the less indispensable part of dystrophin (Figure 3).

DYSTROPHIN AND DMD THERAPY

Indeed, the injection of naked whole cDNA has been proven to be inefficient to produce significant dystrophin levels in diseased muscles and the cDNA has to be vectorised to specifically reach muscles. For this purposes, different serotypes of adeno-associated viruses (AAV) have been used. However, the whole *DMD* cDNA is too large to be inserted in the AAVs. Therefore, the concept of micro-dystrophin has

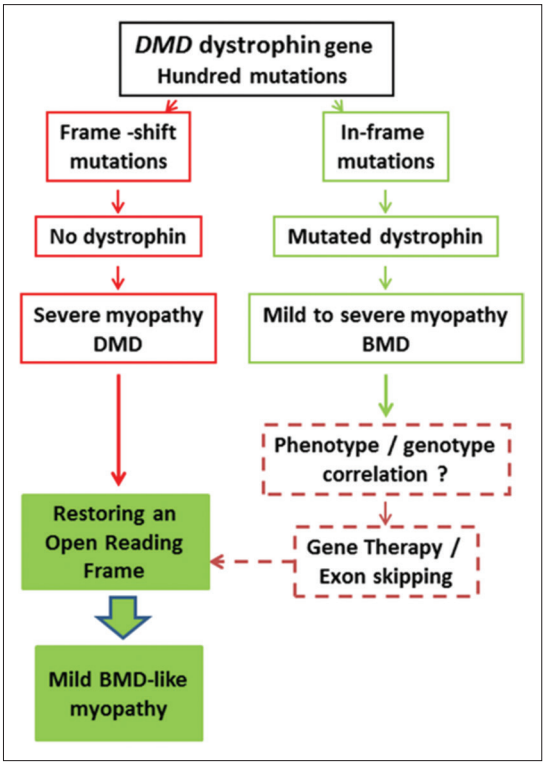


FIGURE 3. The Monaco rule and how it is used to generate therapy patterns.

emerged [52]. Truncated gene coding sequences (micro-dystrophins) inspired from the truncated dystrophin coding sequences observed in mild BMD patients [53](Figure 3) were designed [54-57]. One of these has been well used in animal models such as the *mdx* mouse and the *grmd* dog. This highly simplified micro-dystrophin only consists of the N- and Cys-rich domains with two hinges and four repeats (Figure 4)[58, 59]. However, this micro-dystrophin does not recapitulate all the functions of dystrophin [54] and further improvements are needed essentially by addition of other binding domains of dystrophin such as for example, the nNOS and microtubule binding sites (Figure 2) [56].

The exon skipping therapy aims at recover an in-frame mutation equivalent to a BMD mutation. In case of the frame-shifting deletion of exon, modifying the splicing of mRNA allows the exclusion of one or several additional exons and the restoration of a reading frame (Figure 5). To

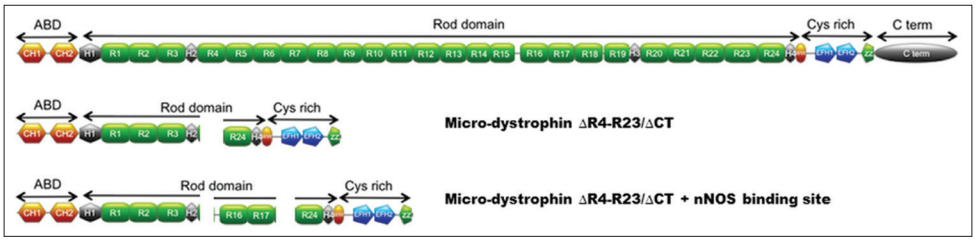


FIGURE 4. Two examples of micro-dystrophins.

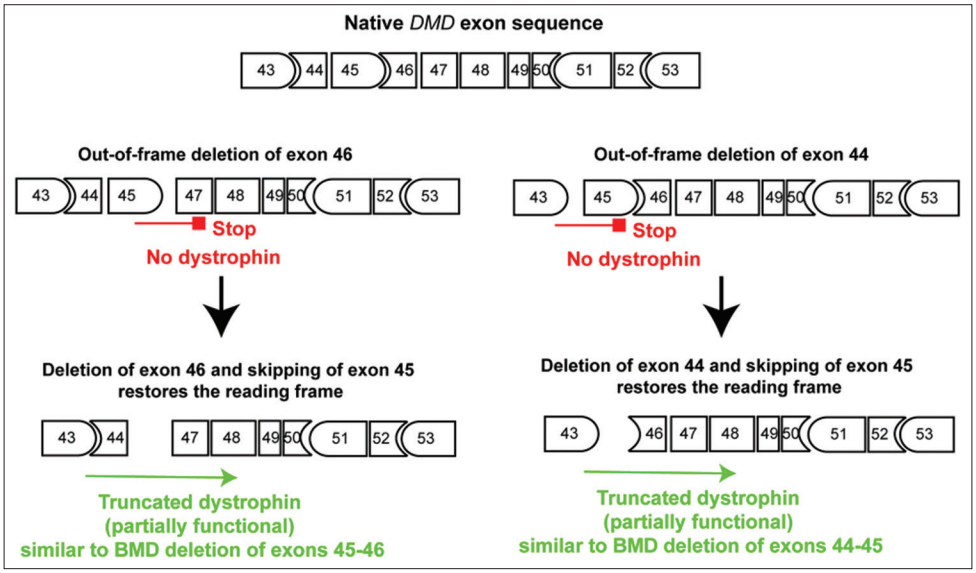


FIGURE 5. Exon skipping for restoration of the reading frame. Same legend as in Figure 1.

this end, small antisense oligonucleotides (AONs) sequences are designed to bind to exon splice junctions, masking them from the spliceosome. This modifies the splicing and skips the exon (s) focused on from the mRNA. Two types of AONs have been designed, 2'O-methyl phosphorothioate (2'O-Me) or phosphorodiamidate morpholino (PMO) oligomers.

The proof of concept has been largely demonstrated [60-66] [67] and there are now human trials in progress using oligonucleotides injection using AAV [68-72](see the excellent reviews about therapy [56, 73-74]). For example, the deletions of exon 46 or 44 both shift the reading frame and result in no dystrophin production. The two deletions can be enlarged by skipping exon 45 resulting in the two in-frame deletions 45-46 and 44-45, respectively (Figure 5). Theoretically, the two enlarged deletions should lead to the production of a BMD-like truncated and partially functional dystrophin.

However, these schemes are more complex than at first glance since a lot of BMD patients have a more severe disease than others. This high variability of phenotypes has long been recognized but rather recently examining BMD patients in details has emerged to be relevant to anticipate for DMD therapy effects. In the two examples here in Figure 5, two BMD-like deletions may be produced using the same drug in different patients. However, the deletion 45-46 has been recently reported to be accompanied by a DMD phenotype [75] and to produce no dystrophin (personal communication). On contrast, there are no patients reported for the deletion of exons 44-45, signifying that the deletion may be asymptomatic. Therefore, the therapy of these two DMD deletions may not result in the same cure effect even though we anticipate that patients will all be transformed in BMD-like patients.

In that field, we recently studied the clinical status of BMD patients with deletions starting by exon 45 deletion e.g. deletions of exons 45-47, 45-48, 45-49 and 45-51 and the ***in vitro*** biochemical status of the proteins [76]. We showed that the structure of the new dystrophins as produced in these BMD patients could partly explain the differences in the clinical severity of the patients. The patients with deletions 45-47 and 45-49 were confined to wheelchair or were diagnosed with a cardiomyopathy about 10 years earlier than patients with deletions 45-48 and 45-51. The new junction at the deletion site of these two last deletions reconstitutes a spectrin-like structure while it is not the case of the two first deletions. Therefore, the choice for skipping one exon or the other for restoring the reading frame to a DMD deletion means that the genotype - phenotype correlation data in BMD patients is well established. In addition, this proves that examining the status of large cohorts of BMD patients together with the status of dystrophin is of high interest for the therapy design. For the clinicians, this also signifies that it is essential to diagnose all the BMD patients by genetics and to follow the time course

of the disease by regularly evaluating respiratory and cardiac muscle status and walking ability of the BMD patients.

CONCLUSION

Finally, it appears that therapies in progress will not cure the DMD disease but slow down its progression. The purpose now is to focus therapy on the BMD-like dystrophin sequences with the less severe disease either for gene or exon skipping therapy. We have now to increase our knowledge about BMD disease genetics and time course and dystrophin structure consequences after BMD deletions. The effects of genetics modifiers have also to be studied in details such as non-synonymous polymorphism (SNPs) [4], noncoding RNAs [77, 78] or other genetic modifiers [79-83] as they all may modulate the expression of dystrophin or the severity of the disease. The diagnosis of BMD is highly relevant to anticipate and to understand data resulting from human clinical trials. It is also likely that certain severe BMD patients should benefit from therapy strategies or from compensatory strategies elaborated for DMD patients.

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