1 Introduction

Introduction

In the mid-1800s reports emerged in literature about a muscle disease affecting young boys.[1] Characteristic about these cases was a progressive muscle weakness, hypertrophy of affected muscles (especially the calves) and development of contractures. Guillaume Duchenne (1806-1875), a French neurologist, described a case series of thirteen of these patients and noted six diagnostic features:

1. Decrease in strength, at the beginning of the disease, usually in the muscles of the lower limbs.
2. Lordosis and spreading of the lower limbs on standing and walking.
3. Excessive development of volume, during a second stage, either of some or all of the weakened muscles.
4. Progressive course of the disease, during a third stage, with worsening of the paralysis and with its generalization if it was limited to the inferior members.
5. Decrease or abolition of electromuscular contractility in an advanced stage of the disease.
6. Absence of fever, sensory disturbance, and impairment of the functions of the bladder and intestine during the entire course of the disease.

Duchenne called the condition "pseudohypertrophic muscular paralysis". In England, Sir William Gowers (1845-1915) independently also examined and described several of these patients and added the distinctive inheritance pattern through the female line which affects almost exclusively boys to the characterization of this novel disease. He noted:

> "The disease of one of the most interesting, and at the same time most sad, of all those which we have to deal: interesting on account of its peculiar features and mysterious nature; sad on account of our powerlessness to influence its course, except in slight degree, and on account of the conditions in which it occurs. It is a disease of early life and early growth. Manifesting itself commonly at the transition from infancy to childhood, it develops with the child's development, grows with his growth-so that every increase in stature means an increase in weakness, and each year takes him a step further on the road to hopeless infirmity and in most cases to an early and inevitable death."

Today the disease depicted by these and other physicians is known as Duchenne Muscular Dystrophy (DMD). In the 1950s, the German neurologist Peter Becker described a similar disease in boys, although the muscle weakness started at an older age and progression was slower.[2] This disorder which later on appeared to be caused by a mutation in the same gene as DMD is known as Becker muscular dystrophy (BMD).

Natural history of DMD and BMD

Symptoms in DMD patients typically present in toddlers with difficulty rising from the floor and a waddling gait. Other early symptoms are the tendency to tiptoe, inability to run, frequent falls and the above mentioned strikingly large calve musculature. Some patients complain about muscle cramps and myalgia. Patients develop difficulty with climbing stairs and increasingly rely on their upper extremities pulling themselves up the stairs. Walking becomes difficult and patients generally become wheelchair

dependent between the ages of 8 and 12 years.[3-10] A scoliosis often develops needing surgery.[4,8,9,11-13] Without treatment, death usually occurs at the late teens, due to respiratory or cardiac failure.[3,5,7-9,13,14] The initiation of home mechanical ventilation in DMD patients has led to an improvement in survival into the twenties and thirties.[5,7,15,16] With these developments in respiratory care, cardiac failure has become the main cause of death.[16] Besides the progressive muscle weakness characteristic for DMD, speech delay is reported for most young patients and approximately one-third of DMD patients also experience non-progressive cognitive impairment.[17] Symptoms can also involve the gastro-intestinal tract with difficulties chewing and swallowing, reflux and constipation.[18]

BMD patients show a milder and more diverse disease course than DMD patients. First symptoms can be noted anywhere from infancy to middle age and common presenting symptoms include calf pains, falling, moving slower than peers, difficulty walking stairs and a waddling gate.[19-24] Asymptomatic patients solely presenting with raised serum creatine kinase levels in routine blood testing are also not uncommon.[25,26] Age at wheelchair dependence ranges from the late teens to the seventies, though many patients remain ambulant throughout life.[19,21,22,27] As all disease parameters, survival of BMD patients shows much diversity, with patients dying in their twenties to their late eighties.[21] Mortality is frequently caused by cardiac failure or arrhythmias, though death due to respiratory failure does occur.[28-31]

Medical care

In recent decades many developments in care for DMD patients have been made. Firstly, since the publication of several studies showing the use of corticosteroids to be associated with a slower decline in muscle strength and function, steroid prescription has become part of standard DMD treatment.[32-34] Several observational studies since have shown steroids to delay age at wheelchair dependence, and suggested that they also decrease scoliosis development and delay the decline in respiratory and cardiac function.[4,12,14,35-38] However, controversy still exists concerning the best dosing schedule for Prednisone (daily or ten-days-on-ten-days-off) taking both efficacy and side effects into account.[39-41] This is the subject of a large multicenter study currently being executed (*NCT01603407*). In this study the possible difference in effect and/or side effects of two forms of steroids, deflazacort and prednisolone, is also investigated.

Another important improvement for DMD patients has been the introduction of mechanical home ventilation. As respiratory failure was a major cause of death in DMD, the use of ventilatory support has significantly improved survival in DMD, adding six to more than ten years.[5,7,40,42] Other developments in care include the prescription of cardiac medication, the use of physiotherapy and splints and Achilles tendon lengthening. These and other developments have transformed DMD from an incurable disease into a chronic multisystem disorder, necessitating good monitoring and follow-up by a multidisciplinary team of specialists.[43]

Genetics

DMD and BMD are both caused by mutations in the *DMD* gene. This gene consists of 79 exons, coding for the dystrophin protein. In general, DMD is caused by mutations that lead to absence of dystrophin.[27] Most DMD patients have a disruption of the reading frame due to deletions or duplications of exons.[44] Other causes for dystrophin absence include point mutations causing a premature stop codon, micro-deletions disrupting the reading frame and splice site mutations leading to the exclusion of an exon from the transcript, indirectly causing a frame shift.

Contrary to DMD patients, BMD patients generally do express dystrophin. BMD mutations typically do not disrupt the reading frame, but allow the production of (reduced quantities of) an internally deleted dystrophin protein.[44]

The dystrophin protein and dystrophin associated glycoprotein complex

Dystrophin is an important part of the dystrophin-associated glycoprotein complex (DCG). This trans-sarcolemmal complex, which besides dystrophin consists of sarcoglycans, dystroglycans, sarcospan, syntrophin and neuronal nitric oxide synthase (nNOS) connects the cytoskeleton of the muscle fiber with the extracellular matrix.[27,45-47] The dystrophin protein consists of four distinct domains (Figure 1&2):

1. The N-terminal domain, which contains binding sites for actin.[48-50]
2. A central rod domain, consisting of 24 spectrin-like repeats and 4 hinge-regions.[51] This domain functions as a buffer zone, enabling dystrophin to lengthen when stretched during muscle contraction.
3. A cysteine-rich domain, which connects to the sarcolemma through binding of beta-dystroglycan.[52-54]
4. A C-terminal domain, which binds syntrophin, another component of the DCG.[52,55]

Through their interactions the components of the DCG complex play an important role in protecting the muscle membrane from contraction-induced injury. Dystrophin, being connected to the contractile machinery of the muscle fiber, plays a key role in this stabilizing function. Therefore, absence (as in DMD) or decreased functioning (as in BMD) of this protein leads to repetitive cycles of muscle damage. Clinically, this ongoing process leads to progressive muscle weakness.

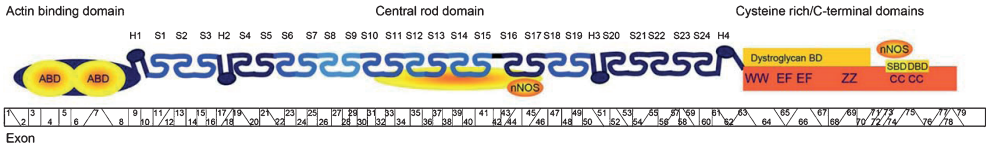

Figure 1. Schematic structure of the dystrophin protein, showing the binding domains for Actin (ABD), nNOS and dystroglycan.

The central rod domain consists of 24 spectrin-like repeats (S1-S24) and 4 Hinges (H1-H4). Just below the color figure of the dystrophin protein the exons coding for the specific regions are depicted.

Picture by A. Aartsma-Rus.[145]

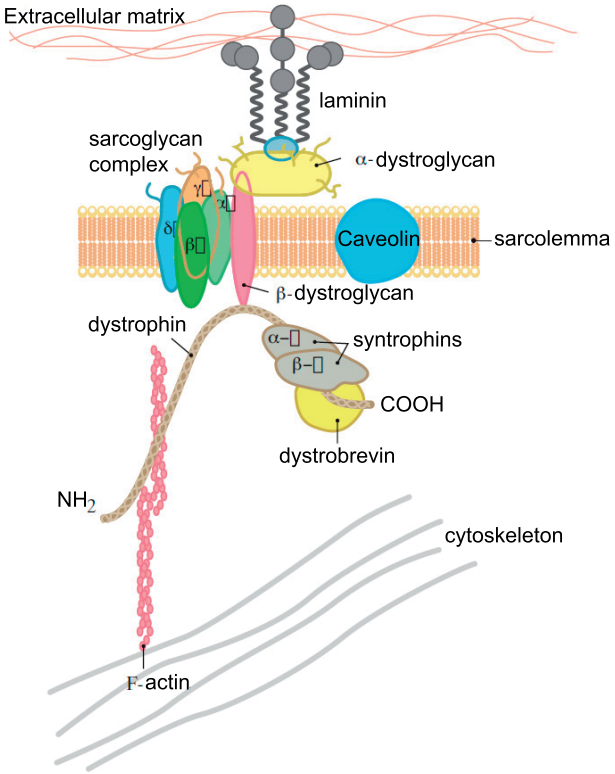

Figure 2. The dystrophin-associated glycoprotein complex, with its interactions with F-actin and the extracellular matrix.
Picture by J. den Dunnen.[146]

Diversity in disease course

As mentioned previously, there is much diversity in disease course for BMD patients. To a lesser extent, DMD is also characterized by variability in disease progression. This can be noted when looking at the age at loss of ambulation: while some patients remain ambulant until 12 years, others become wheelchair dependent before the age of 8.[56] Little is known about the causes of these differences in both DMD and BMD, but there are several factors that have been implied.

Mutation

As the dystrophin protein forms a connection between the contractile elements of the muscle fiber and the extracellular matrix, it could be expected that some mutations lead to a more severe disruption of dystrophin's function than others. In general, this applies to mutations causing DMD versus mutations causing BMD: mutations disrupting the open reading frame cause a DMD phenotype, while a maintained open reading frame leads to BMD. Also within the spectrum of BMD phenotypes there

appears to be a role for the site of the mutation in the *DMD* gene, influencing the quality of the dystrophin protein produced. Mutations in the cysteine-rich domain are the most severe and cause a DMD phenotype, while mutations involving the N-terminal domain are known to present with a severe BMD phenotype.[22,25,57,58,58-61] Since the actin binding (N-terminal) domains and the dystroglycan binding (cysteine-rich) domain are crucial for dystrophin function, it is not surprising that mutations abolishing all actin binding domains or the dystroglycan binding domain lead to non-functional dystrophin and are associated with serious contraction-induced injury and therefore a severe disease course.[62] In general, mutations involving the central rod domain lead to a milder often called 'typical' BMD-phenotype.[22,63-66] Apparently, this part of the dystrophin protein is mostly dispensable.[24,67,68] However, very large mutations deleting most of the spectrin-like repeats have been shown to cause a more severe phenotype.[69-72] Several case reports have also implicated the way the phasing of the spectrin-like repeats are disrupted to influence disease severity: the deletion of one or several complete repeats would result in a milder disease course than mutations that cause the partial deletion of a repeat.[63,73-75] Involvement of hinge regions of the rod domain or the spectrin-like repeats flanking these regions possibly cause a more severe disease course, as this influences the flexibility of the dystrophin protein when strained.[73-75] A last observation influencing the disease severity of mutations involving the central rod domain is the occurrence of another actin binding site within this region.[23,76] Mutations involving this region but leaving the N-terminal actin-binding domains intact are associated with a more severe disease course.[77] Finally, mutations involving only the C-terminal domain are rare. However, case reports involving patients with these mutations show the disease course to be relatively mild.[78-80] Research in *mdx* mice, a mouse model for DMD that lacks full-length dystrophin, shows that expressing dystrophin that is deleted for this C-terminal domain present with a mild disease course, which is in line with the observation in humans.[52,81] Although the general rule states that out-of-frame mutations lead to a DMD phenotype, there are some exceptions. Firstly, it has been well established that frame-disrupting mutations involving the exons 3 to 7 can present with a BMD phenotype.[17,44,58,70,72,82-89] Studies in muscle biopsies of these patients have proven that these patients do produce dystrophin. There are two possible explanations for this observation:[85]

1. *Use of an alternative translation initiation*: Normally, translation is initiated in exon 1 of the dystrophin transcript. However, mutations before exon 8 can activate alternative translation initiation sites present in exon 6 and 8 and in that way bypass the out-of-frame RNA-transcript. [90] The resulting dystrophin will lack most of the N-terminal domain. The efficiency at which these alternative translation initiation sites are activated varies between patients.

2. *Spontaneous exon skipping*[82,83]: This occurs when pre-RNA is converted to messenger RNA (mRNA) by the splicing of introns. In patients with a deletion of exon 3 to 7 sometimes exon 8 is also excluded during the formation of mRNA.[58] Therefore the deletion of the patient is enlarged from exon 3 to 7 to a deletion of exon 3 to 8, restoring the RNA reading frame and enabling the production of (internally deleted) dystrophin. Alternatively, spontaneous skipping of exon 2, leading to a deletion of exon 2 to 7, also restores the reading frame.[58]

Other mutations have also been found to be exceptions to the reading-frame rule, among which are single exon deletions of exon 45 and 51, several mutations at the 5'-end of the *DMD* gene, deletion of exon 42 and 43 and deletion of exon 48 to 50.[74,86,87,89,91-93] BMD patients with an out-of-frame deletion of exon 45 have been found to spontaneously skip exon 44, restoring the reading frame. However, in some of the reports the potential discrepancy might be the result of inaccurate genotyping, when for instance DNA-diagnostics is performed by only multiplex PCR, which does not evaluate the presence of all exons. Mutations could therefore erroneously be described as out-of-frame, while in fact the mutation is in-frame. As can be concluded from these data, the site of the dystrophin mutation appears to be an important factor determining disease severity. However, even when comparing patients with the same mutation there is diversity in disease course.[83,94] For example, BMD patients with a deletion of exon 45 to 47 all show a relatively mild disease course, but even within this genetically homogeneous group variability exists, with some patients becoming wheelchair dependent in early adulthood, while others are still ambulant in middle age (Table 1).[25,72] Even BMD patients from one family can present with different degrees

Table 1. Clinical information of BMD patients previously described in literature with a deletion of exon 45 to 47.

Current age	Age first symptoms	Age at status Asymptomatic	Age at status Mild	Age at status Moderate	Age at status Severe	Age at wheelchair dependence	Reference
66	33						106
64							72
62	23					59	106
61	30	-	30	45	55		22
59	30						106
52							72
47	28						106
42	15	-	15	27	42		25
40							72
36	17						106
35	13	-	35				22
35							72
35							72
34	5	-	24	34			22
34							72
33	11	-	11	25			22
33							72
32	8	-	8	20			25
32	10	-	20				22
32	14	-	32				22
29	8	-	8	29			25
29	13						106
29							72
28	15	-	15	21			25
28							72
27	3	-	8	19	27		22

Table 1. Clinical information of BMD patients previously described in literature with a deletion of exon 45 to 47. (continued)

Current age	Age first symptoms	Age at status Asymptomatic	Age at status Mild	Age at status Moderate	Age at status Severe	Age at wheelchair dependence	Reference
27	7	7	18	27			25
27							72
26	10						106
25							72
24	10						106
23	10	-	20				22
22							72
19							72
19							72
18							72
18							72
17	6	-	6	14			22
17		-			17		25
17	16	-	17				22
16	1.5						106
16	6						106
16							72
16							72
15	3						106
15							72
15							72
15							72
13	12	12		13			25
12	3	-		3	12		25
10	4	-	4				25
10	4.5	-	8				25
10							72
8	8	-		8			25
8	8						106
5	3	-	3				25
4	2	-	2	4			25
4	3	-	3				25
4	4						22
unknown						21	72
unknown						30	72
unknown						30	72

Asymptomatic: elevated serum creatine phosphokinase (CPK) and/or calf hypertrophy and/or cramps;
Mild: fatigue and/or any detectable weakness including reports of "clumsiness", falling, abnormal gait, toe walking, and slow running, all in the absence of a positive Gowers's sign;
Moderate: positive Gowers's sign, difficulty with stairs, and/or waddling gait;
Severe: inability to rise without assistance and/or ≤3/5 strength in major proximal muscle groups and/or ambulation only with effort and/or severe muscle wasting of muscles.
Grey boxes indicate no information being available about that specific parameter in that patient.

of muscle weakness.[22,95-100] As these patients have an identical mutation, this intrafamiliar variability provides evidence that mutation in the *DMD* gene is not the only factor involved in disease severity.

Dystrophin levels

One of the possible other factors involved in disease severity is the quantity of dystrophin expressed in muscle fibers. It is generally accepted that dystrophin levels of 3% or less lead to DMD.[27,101,102] Some studies suggest that even within the DMD spectrum, dystrophin expression influences disease severity: expression of some dystrophin would be associated with a milder disease course, as measured by age at wheelchair dependence, than expression of no dystrophin at all.[102,103] For BMD patients there is controversy about the relationship between dystrophin quantity and disease severity. Most studies state that more dystrophin is associated with a milder disease course, claiming a dose-effect relationship.[10,19,22,56,87,101,102,104-106] On the other hand, a study by Hoffman et al points towards a threshold effect, where dystrophin levels below approximately 10% are associated with a severe disease course, while the effect of higher dystrophin levels on disease severity is not that evident.[107] Other studies failed to find any relationship between dystrophin expression and BMD phenotype.[25,64]

Besides the amount of dystrophin produced, some studies also point towards a role of the nature of the dystrophin expression pattern. They state that DMD patients expressing some level of dystrophin benefit more clinically, if there is mild expression in all muscle fibers instead of moderate/good expression in some fibers, while other fibers show no dystrophin at all.[103,105]

Dystrophin-associated glycoprotein complex

Dystrophin connects the contractile machinery of the muscle fiber with the extracellular matrix, and in that way contributes to the stability of the sarcolemma. One could imagine that reduced expression of other proteins involved in this interaction (components of the DCG) would also influence the occurrence of contractile induced injury and therefore contribute to disease severity. Indeed, several proteins of the DCG are themselves associated with muscular dystrophy of various severities.[108,109] In DMD, the components of the DCG are mostly absent or severely reduced, illustrating the importance of dystrophin for correctly localizing these proteins.[110-112] In BMD, expression of the other components of the DCG is variable.[113,114] As the complex is involved in muscle-membrane stability, reduced quantity of components of this complex could influence disease severity in BMD. Indeed, a study by Anthony et al showed asymptomatic BMD patients to have higher levels of beta-dystroglycan than mildly affected patients.[104] This study also investigated the role of alpha-sarcoglycan expression, but no correlation with disease severity was found. They postulate that possibly only proteins binding directly to dystrophin influence disease severity.

Besides its role in stability of the muscle membrane, the DCG is also involved in signaling. One of the best studied components involved in this secondary function of the DCG is neuronal nitric oxid synthase (nNOS). This protein binds to dystrophin both directly, at the central rod domain, and indirectly, through interaction with syntrophin at the C-terminal domain.[115-118] It is responsible for the production of Nitric

Oxide (NO), which plays an important role in functional sympaticolysis: the inhibition of alpha-adrenergic vasoconstriction when muscles contract.[119] This process facilitates the transport of sufficient amounts of oxygen and nutrients to the contracting muscle. Absence of nNOS therefore is expected to lead to muscle ischemia. It is thought that in dystrophic muscle, ischemia causes permanent muscle damage through the occurrence of muscle edema and in this manner contributes to disease severity.[120-122] Indeed, two studies that investigated nNOS expression in BMD both showed high levels of nNOS at the sarcolemma to be associated with a milder disease course.[104,123]

Inflammation

The previously described factors all involve the organization at the sarcolemma. While these are the most obvious factors contributing to disease severity in the dystrophinopathies, more general factors could also be of interest. One of these factors is inflammation. As muscle damage occurs, an inflammatory response is initiated. This response is necessary to remove debris, enabling regeneration of the muscle fiber. However, in dystrophic muscle the inflammatory response endures and becomes part of the disease process, causing more muscle damage and leading to fibrosis. Indeed, studies in DMD muscle tissue have shown diverse inflammatory factors to be upregulated early in the DMD disease course and to remain at this higher expression level during at least childhood.[124-127] Diminishing this inflammatory response could be the mechanism of action explaining the beneficial effect of corticosteroids on delaying disease progression in DMD patients, although other possible steroid effects have also been implicated.[128]

Differences in the innate immune response in dystrophinopathy could be one of the factors involved in variability in disease severity. Several studies support this notion, by demonstrating that a single nucleotide polymorphism (SNP) in the secreted phosphoprotein 1 (*SPP1*) gene contributes to diversity in disease progression in DMD patients.[129,130] The *SPP1* gene codes for the osteopontin protein, which is believed to have pro-inflammatory effects in muscle.[131,132] The SNP is located upstream of the *SPP1* promoter, influencing expression levels of osteopontin, and could therefore have an impact on the inflammatory response in DMD muscle.

Fibrosis

The chronic inflammatory response inhibits the continuous cycles of degeneration and regeneration accompanying the DMD and BMD phenotype. Due to the cytokines produced chronically by the inflammatory cells, fibrosis formation is initiated. Fibrotic tissue itself also inhibits muscle regeneration, thus further contributing to the pathology. Eventually these processes lead to the loss of muscle tissue and replacement by fatty and fibrous tissue. Factors influencing this adverse response to muscle damage could be involved in the variability of disease progression. A study by Flanigan et al found an association between four SNPs in the latent transforming growth factor 4 (*LTBP4*) gene and age at loss of ambulation in DMD.[133] These SNPs lead to different amino acids being built into the LTBP4-protein, making up different haplotypes. IAAM, the *LTBP4* haplotype that in this study was associated with a prolonged

ambulation is related to decreased TGF-β signaling. As TGF- β is an important regulator of fibrosis and inhibits satellite cell differentiation, this haplotype could indeed be associated with less fibrosis and therefore a milder disease course.[134-136] Research also implicated osteopontin to be involved in the fibrotic process in DMD, adding to the possible mechanisms by which the *SPP1* SNP influences disease severity.[132] This study also showed that several tissue remodeling markers (Matrix Metalloproteinase 2 and 9 and TIMP Metalloproteinase Inhibitor 1) were upregulated in muscle of DMD patients, compared to normal human muscle or even muscle from BMD patients.

Development of possible treatments

While optimal care increased the survival and quality of life of DMD patients significantly in the past decades, a market authorization for a DMD treatment has been long in coming. As many possible therapies are under investigation, I here limit the information to treatment options that are currently in the late phases of clinical trials.

Recently, the first treatment aiming to restore dystrophin production in DMD patients has received conditional market authorization by the European Medicines Agency (EMA) for use in ambulant DMD patients aged 5 years or older. This drug, Translarna, could be beneficial to the approximately 13% of DMD patients with a nonsense mutation in the *DMD* gene leading to a premature stop codon. Translarna enables ribosomal read-through of these stop codons and can therefore restore dystrophin production in these DMD patients. A phase-2a trial (*NCT00264888*) in young DMD patients indeed showed a mean increase in dystrophin levels of 11% (measured using the dystrophin/spectrin ratio by immunohistochemical analysis) in 65% of treated patients, while a phase-2b study (*NCT00592553*) of 48 weeks in 174 patients showed a significant improvement on the six minute walk test (6MWT) for treated patients compared to placebo.[137] The 6MWT measures the distance walked at a self-chosen pace in 6 minutes, and usually measures the difference between the distance walked at baseline and a preset timeframe, generally six months or one year. Currently, a phase 3 trial (*NCT01826487*) is fully recruited, aimed at confirming the beneficial effect of Translarna, enabling the conditional market authorization to be altered to full marketing authorization in the future.

Another treatment under development is the exon skipping technology. This RNA-centered approach aims to change an out-of-frame transcript into an in-frame transcript by using small pieces of modified DNA (antisense oligonucleotides) to hide a specific exon (or exons) from the splicing machinery during pre-mRNA splicing. This exon is subsequently not included into the mature messenger RNA-transcript leading to restoration of the reading frame and enabling dystrophin production. Several studies have already shown this approach to result in restoration of dystrophin production, after local injection and systemic delivery of antisense oligonucleotides targeting exon 51, and therefore aiming to exclude that specific exon from the mRNA-transcript[138-140] Clinical trials of two different chemically modified antisense oligonucleotides (Eteplirsen (*NCT01396239*) and Drisapersen (*NCT01462292*)) showed positive results on the 6MWT when treatment was started in a relatively early phase of the disease.[141,142] A large phase 3

study of Drisapersen (*NCT01254019*) involving 186 ambulant DMD patients failed to show a significant or clinically meaningful difference in the results of the 6MWT. It was suggested that the lack of a positive effect in this trial was caused by the older age and concomitant more advanced disease of participants at start of the trial.

While Translarna, Eteplirsen and Drisapersen use mutation specific approaches, drugs that could be beneficial to all patients are in development as well. For cardiac and respiratory function, research is performed into a possible disease modifying effect of Idebenone. This drug has anti-oxidative qualities and improves mitochondrial respiratory chain function and in that way influences a cell's energy production. A phase 2 study in 21 DMD patients (*NCT00654784*) showed a trend towards a beneficial effect of this drug on both cardiac and respiratory function.[143] Recently, results of a phase 3 trial (*NCT01027884*) were presented, showing a significant effect in delaying the loss of respiratory function for DMD patients between 10 and 18 years of age treated with Idebenone compared to placebo in patients not using corticosteroids at time of study.

Tadalafil is a phosphodiesterase 5A (PDE5A) inhibitor that increases the half-life of cyclic guanosine monophosphate (cGMP), a downstream target of nNOS located in the vascular smooth muscle. By inhibiting the breakdown of cGMP, Tadalafil might prevent exercise induced vasoconstriction of the vascular musculature and in that way prevent muscle ischemia. A recent study indeed showed a positive effect of Tadalafil on oxygenation and blood flow in exercised forearm musculature of DMD patients.[144] Currently, a phase 3 trial (*NCT01865084*) is being executed investigating the possible effect of Tadalafil on slowing disease progression in DMD.

Although the above mentioned therapeutic strategies appear promising and an approved drug is now available for some DMD patients, for most patients currently no treatment is available. Further developments in this field build on the presence of detailed natural history data, knowledge about pathophysiological processes and increase of insight into factors involved in disease severity.

Aims of this thesis

This thesis aims to describe the disease course of the Dutch DMD and BMD population. Furthermore, it focusses on the investigation of possible factors involved in variability in disease severity in both DMD and BMD patients.

In **chapter 2** the disease course of the Dutch DMD population born between 1980 and 2006 is described. Data are presented about age at diagnosis, wheelchair dependence, scoliosis surgery, start of mechanical home ventilation and death. Information about use of corticosteroids is also provided. To evaluate the effect of changes in care, these data are compared to a Dutch historical cohort born between 1961 and 1974.

Chapter 3 provides clinical information about DMD patients with mutations that are amenable for skipping of exon 44, 45, 51 and 53 respectively, showing that patients with a mutation amenable for exon

44 skipping present with a milder disease course (measured by age at wheelchair dependence) than the other patients.

Chapter 4 focusses on the disease course of Dutch BMD patients with a mutation leading to the production of a truncated dystrophin protein that equals the dystrophin protein that would be produced in DMD patients by successful skipping of five different exons.

Chapter 5 entails the development of a disease severity scale in BMD patients, enabling a better comparison of disease severity among BMD patients of different ages.

The role of dystrophin quantity in disease severity is subject of **chapter 6**. In this chapter, dystrophin levels of BMD patients with different clinical severities are compared.

In **chapter 7** the influence of other components of the DCG on disease severity in BMD are investigated.

Chapter 8 involves the validation of the SNPs in the *LTBP4* and *SPP1* gene as markers of disease severity in DMD patients. The main results and conclusions are discussed in **chapter 9.** Lastly, **chapter 10** provides a summary of this thesis in English and Dutch.