9 Discussion and future perspectives

Discussion

The dystrophinopathies have been the subject of much research in recent decades. Advances have been made in understanding the pathophysiology of BMD and DMD, in knowledge of the natural history and in the development of possible treatments. This thesis has added to these advances, by providing information about the disease course of specific genetic subgroups of DMD and BMD patients, the effect of developments in care for DMD patients and about the involvement of certain factors in disease severity. However, a lot is still unknown about both DMD and BMD and much research still has to be performed. Some general points are important to facilitate this research.

Registries

Clinical research projects are generally only possible with the assistance of patients. Some DMD/BMD studies (for example research into possible biomarkers for disease progression) need large numbers of patients, while others need patients with specific characteristics (like steroid status, a certain age or mutation). Finding these research participants is difficult due to the fact that the dystrophinopathies are rare muscle disorders, affecting only 1:4700 (DMD) and 1:18000 (BMD) new born boys.[147,199] This underlines the importance of (international) collaborations, as initiated by the TREAT-NMD alliance. This worldwide network aims to provide an infrastructure 'advancing diagnosis, care and treatment for those living with neuromuscular diseases around the world' and consists of clinicians, academic researchers and patient representatives. It is involved in the construction of standards of care, development of networks of possible clinical trial sites and the development of patient registries for several neuromuscular disorders, among which the dystrophinopathies.[234] Through these (national) databases the recruitment of suitable patients for research projects can be facilitated.[235] Much of the research presented in this thesis has been made possible by use of the Dutch Dystrophinopathy Database, which is part of the TREAT-NMD dystrophinopathy registry. In this way data about epidemiology, natural history and the effect of developments in care in the Netherlands were provided and recruitment of BMD patients for our study into possible factors involved in disease severity was achieved.

Another international collaboration facilitating research into the dystrophinopathies is BIO-NMD. This spin-off project of TREAT-NMD aimed to discover and validate biomarkers in muscular dystrophy with focus on improving disease and therapy monitoring. A biomarker is an objectively measurable indicator of a biological or pathological process or the response to a specific therapeutic intervention.[236] Biomarkers can assist in the diagnostic process (e.g. ASAT and ALAT levels in liver disease), assist in predicting the prognosis or estimating disease severity (e.g. creatinine levels in kidney disease) and be used to evaluate the effect of therapies (e.g. PSA-levels in prostatic cancer). When searching for possible biomarkers two techniques can be used. First of all, one can screen many possible factors that could function as a biomarker at once and see if any of them correlate with the occurrence or severity of a specific disease. This non-targeted approach requires a large number of patients, as correction for multiple testing should be performed. Alternatively, one can specifically investigate one possible biomarker, which has

previously been implicated or theoretically could be involved in a disease. Although this requires fewer specimens than the non-targeted approach, one could strengthen this technique by the use of larger quantities of specimens, enabling discovery of biomarkers with a smaller disease modifying effect. By international collaboration BIO-NMD provided biomaterials (like DNA and muscle tissue) of a relatively large group of patients to research projects involved in biomarker studies in muscular dystrophy.[104,236] In this thesis we describe one such study executed using DNA from five partners involved in BIO-NMD, which investigated the role of SNPs in the *SPP1* and *LTBP4*-genes in influencing disease progression in DMD. Without this collaboration, patient numbers would have probably been too low to execute these analyses. The BIO-NMD project has also been able to identify several protein biomarkers in serum that differentiate between DMD patients and healthy controls and even between DMD and BMD patients.[237] Overall, databases like the DDD and BIO-NMD provide important resources into a better understanding of the pathophysiological mechanisms involved in DMD and BMD, assist in the diagnostic process and facilitate research into possible treatments. Efforts should therefore be made into inclusion of as many patients as possible into these databases and expanding the development of databases to other rare diseases.

Outcome measures

When performing research in DMD or BMD patients, it is important to have adequate outcome measures. For DMD, observational studies often use the age at wheelchair dependence as a measure of disease severity. The advantage of this method is that it does not involve actual measurements being done in the participants; a questionnaire can suffice. It therefore is a cheap method that can be applied in large numbers of patients. However, it can only be applied in older patients, where at least the majority has lost ambulation. An alternative method involves repeated measurement of some functional parameter, like handgrip or time to stand up from a supine position, and then to compare the decline or incline of this specific parameter in time between different participants. Although this method is more costly and time consuming, it most probably is able to detect smaller differences between patients or patient groups than when only investigating age at wheelchair dependence. This method is applied in trials for possible DMD treatments, where change on the 6MWT is commonly used as primary outcome measure.[141] The 6MWT had already been used by trials into a possible treatment of another disease involving muscle tissue, Pompes disease and was therefore introduced in clinical DMD research. In DMD it proved to be a reliable measure that shows good correlation with other functional tests like the Timed Function Tests and the North Star Ambulatory Assessment and reasonable correlation with quality of life questionnaires.[238-240] Contrary to Timed Function Tests the 6MWT includes some assessment of endurance, an important aspect involved in ambulatory functioning. Another benefit of using the 6MWT in DMD research, is its sensitivity to change, being able to detect a significant difference over a one year period, with a calculated Minimal Clinically Important Difference of 30 meters.[239] Observational studies have also shown that a result on the 6MWT <325m is predictive for a fast decline in ambulatory capacity, indicating a relationship between the result on the 6MWT and loss of ambulation.[238] Therefore,

the 6MWT can be considered as a reliable measurement with clinical significance, making it a useful endpoint in clinical trials.

The advantage of using a method like the 6MWT instead of age at loss of ambulation is to be able to investigate the possible effect of a therapy within a fixed and relatively short timeframe. Using age at loss of ambulation as an outcome measure would imply a relatively long follow-up, as age at wheelchair dependence, even without treatment, can range from anywhere between 7 to 13 years. However, use of the 6MWT only enables research in young, ambulant patients and the loss of ambulation of one participant during a trial can have major consequences on the outcome. This necessitates careful patient selection and the need to define a suitable alternative outcome measure in non-ambulant patients. Recently, a new outcome measure for DMD patients was developed which focussed solely on upper body strength, the Performance of the Upper Limb module (PUL).[241] This new scale would be suitable for trials in both ambulant and non-ambulant DMD patients.

For research in BMD patients things are even more complicated. As the prevalence of BMD is even lower than the prevalence of DMD, recruitment of patients is more difficult. Combining this with the large variability in disease severity makes the choice of good outcome measures challenging. Ideally, BMD research would be performed in a group of patients in the same age group, where differences in disease severity could simply be assessed by the age at reaching specific disease milestones (like wheelchair dependence) or by analysing the increase or decrease of a specific functional test (like the 6MWT) over a fixed period of time, indicating the speed of disease progression. However, such homogeneous groups of BMD patients are not easily available. In this thesis, a disease severity scale for BMD patients that is non-age specific was developed, enabling comparison of BMD patients of different ages. This scale can be used when examining factors involved in determining disease severity. In trials, it is not suitable as an outcome measure, as the different milestones used are commonly too far apart in time to show differences in disease progression in a short timeframe.

Use of corticosteroids

In recent decades, the prescription of corticosteroids has become part of standard care for DMD patients. The use of corticosteroids is associated with many side effects, including weight gain, small stature, fractures, behavioural difficulties like hyperactivity, cataract and difficulties sleeping.[4,35-37,39,242-244] These side effects are one of the main reasons patients (or parents) decide against use of corticosteroids or decide to terminate steroid use prematurely. A recent survey of the Cooperative International Neuromuscular Research Group showed 24% of their cohort of 340 DMD patients between 2 and 28 years to have never used steroids, while in an American study in both DMD and BMD boys 47% of 5 to 9 year olds were steroid naive.[245,246] The natural history study described in this thesis found 17% of DMD patients born between 2000 and 2005 to be steroid naïve, while 10% had already terminated steroid use. As can be concluded from these numbers, there is still a substantial group of DMD patients not using steroids. Even among steroid users there are differences, as corticosteroids are prescribed both as prednisolone and deflazacort and several dosing schedules exist. Currently, there is no consensus about the optimal

corticosteroid treatment and research is executed to investigate this further.[39-41] As long as steroid use is not universal and treatment varies, corticosteroids should be considered an important confounding factor in DMD research. Although using steroids as a covariate in analyses or stratifying for steroid use in trials is able to circumvent most of the problem, differences in regimes or dosage might still influence results of clinical DMD research projects. Until the results of the international study into these factors are available, this should be taken into account when planning clinical studies and analysing trial results.
In conclusion, research into the dystrophinopathies warrants international collaboration to obtain sufficiently large study populations and to be able to include subsets of rare patients with specific inclusion criteria. When planning a research project attention should be paid to the assignment of the right outcome measures and the minimization of the possible confounding effects of steroid use.

Future perspectives

Natural history data are important for the understanding of diseases like DMD and BMD and provide important information about standards of care, as also described in this thesis. Although many facts are already known about their disease course, there is still a profound lack of detailed knowledge of several aspects of the dystrophinopathies. Research has largely been directed at symptoms that relate directly to the decreasing muscle strength, like loss of ambulation and mechanical home ventilation. Other aspects, like cognition or bowel function, might have been underrepresented. With the increasing life expectancy of DMD patients, focus has somewhat shifted to include these often disabling symptoms in clinical research. Feeding difficulties is one of these factors under investigation. An Italian study in 118 DMD patients collected data about the occurrence of problems with eating and found 20% of DMD patients to have difficulties swallowing, while 18% report choking.[18] It was recently reported that the dysphagia in DMD is at least partly the result of ineffective tongue movements during swallowing.[247] These developments in the understanding of feeding difficulties can result in an increased awareness of this problem and therefore to better care and possibly treatments. Databases like the DDD are important resources to investigate such secondary symptoms. Future research should investigate the occurrence of other possible problems, like urinary tract symptom (including incontinence for urine), constipation, speech and sexual dysfunction. Knowledge about the prevalence and nature of these symptoms can further guide research into pathophysiological mechanisms and possible treatments. As long as curative treatments are not generally available, attention to optimal care for these patients is of great importance. Observational data like those presented in chapter 2 of this thesis provide strong evidence concerning the protective effect of steroid use on delaying age at wheelchair dependence. Data concerning the effect of steroid use on other aspects of DMD disease progression like scoliosis, cardiomyopathy and respiratory function are less elaborate/definite, consisting mostly of smaller scope retrospective studies.[4,12,34-38,148-150] The use of databases could be beneficial to investigate this possible relationship between steroid use and the progression of these aspects of DMD, enabling comparison of larger groups of patients. Currently, the population of DMD patients on steroids in the DDD is still young, but with adequate

follow-up, information about the occurrence and extent of spinal, cardiac and respiratory involvement in this group of patients will become available. These could provide valuable prospectively collected data concerning the effects of corticosteroids, which are of major importance to inform patients and parents about the clinical efficacy and long-term side effects. This important information is needed to make a balanced decision about the dose and duration of steroid therapy in DMD, especially because it is currently not yet routinely continued after loss of ambulation.

In this thesis the development of a disease severity scale in BMD is described. This scale could be of value in BMD research. However, validation of the scale needs to be performed: do BMD patients maintain the same disease severity Z-score throughout life? For this purpose, the BMD cohort interviewed for the current study should be re-interviewed in several years to obtain a new Z-score to be compared to the present Z-score. Alternatively, our study could be executed in a new population of BMD patients to investigate if similar mean disease scores per age will be obtained.

A disadvantage of our disease severity scale is the retrospective nature of data gathering. Not only does this make the results less accurate, it also limits the items used, since patients might not remember the age at reaching every possible milestone. A prospective data collection concerning disease course would enable the inclusion of more items in our disease severity scale. Possible items could include age at inability to run or to rise from a chair without use of arms, age at inability to walk more than 100 meters without support and age at inability to raise both arms over the head. The adding of these and/or other items will strengthen the scale and will make comparison of individual BMD patients even more accurate.

Besides use of the disease severity scale for research into pathophysiological mechanisms, the scale could also be used in clinical trials. Currently, the development of therapies in BMD is difficult, due to the large variability in disease course. This hinders the formation of comparable research groups. By stratifying BMD patients based on their score on the disease severity scale, this problem could be decreased. This would enable research projects into possible treatment options. Recently, a new therapeutic approach has been proposed in BMD: follistatin gene therapy, aiming to express the follistatin gene in muscle tissue of BMD patients using an adeno-associated virus. Follistatin is a strong myostatin antagonist and as myostatin inhibits muscle growth and differentiation, its inhibition is thought to lead to increased muscle mass and therefore muscle strength. A recent proof-of-principal study (*NCT01519349*) using local injection of the follistatin-vector in quadriceps muscle showed improved distance of the 6MWT in 4/6 BMD patients.[248] Future placebo-controlled studies could best be executed using an as homogeneous BMD study population as possible. The disease severity scale presented in this thesis could assist in selection of these patients.

The relationship between dystrophin quantity and disease severity has been the subject of an extensive amount of research. Although a relationship obviously exists, as DMD patients without dystrophin present with a more severe disease course than BMD patients with reduced quantities of (truncated) dystrophin, the nature of this relationship is still unknown. In this thesis a study is described that reports no linear relationship between clinical weakness and amount of dystrophin. Thus the relationship could

be more complex, including the possibility of a threshold effect. Further research into this possible relationship is currently being executed, investigating the disease severity of a group of patients with dystrophin levels below 20%. Knowledge of this role of dystrophin is of great importance for the current developments in therapies aiming to restore dystrophin production, as it provides evidence to the level of dystrophin necessary to significantly improve disease course.

Much interest exists for the role of nNOS in disease severity. Although in this thesis no relationship between nNOS expression at the sarcolemma and disease severity in BMD patients was found, other studies did.[104,123] Currently, a trial (*NCT01865084*) is being executed in DMD patients investigating the effect of influencing processes secondary to the function of nNOS. Hopefully, this will shed more light on this possible contributing factor and will provide a new therapy.

The role of other components of the DGC has not received much attention. Few studies have investigated their expressional levels in relation to disease progression. The study presented in this thesis did not find a role for beta-dystroglycan and gamma-sarcoglycan, while another study investigating beta-dystroglycan did.[104] Further research into the DGC is therefore warranted.

The strong collaboration of clinicians and basic scientists in the field of the dystrophinopathies, including the development of patient registries, offers good perspectives to further increase the understanding of the disease processes involved. Due to all new techniques that have become available, like the investigation of RNA-expression in muscle tissue, SNPs in DNA and expression of certain proteins in muscle and/or blood, optimism exists that the pathophysiological picture will become increasingly clear, showing the extent to which factors like inflammation, fibrosis, muscle regeneration and oxidative stress contribute to the complex pathogenesis of the dystrophinopathies. This increasing knowledge of the dystrophinopathies hopefully leads to improvement of existing and development of new therapeutic opportunities.